

AMERICAN JOURNAL OF BOTANY

Official Publication of the
BOTANICAL SOCIETY OF AMERICA

EDITORIAL COMMITTEE

RALPH E. CLELAND, Editor-in-Chief
Indiana University

ARTHUR J. EAMES
Cornell University

EDMUND W. SINNOTT
Yale University

RONALD BAMFORD, Business Manager
University of Maryland

JOHN S. KARLING, Secretary
Columbia University

W. J. ROBBINS
New York Botanical Garden

Representatives of Sections of the Botanical Society

CHARLES THOM, *U. S. Bureau of Plant Industry*
General Section

CHESTER A. ARNOLD, *University of Michigan*
Paleobotanical Section

E. N. TRANSEAU, *Ohio State University*
Physiological Section

EDGAR ANDERSON, *Missouri Botanical Garden*
Systematic Section

Representatives of Societies

A. A. DUNLAP, *Texas Agricultural Expt. Station*
American Phytopathological Society

SANFORD S. ATWOOD, *Cornell University*
Genetics Society of America

OTIS F. CURTIS, *Cornell University*
American Society of Plant Physiologists

STANLEY A. CAIN, *University of Tennessee*
Ecological Society of America

L. R. HESLER, *University of Tennessee*
Mycological Society of America

F. W. PENNELL, *Academy of Natural Sciences of Philadelphia*
American Society of Plant Taxonomists

R. M. SMOCK, *Cornell University*
American Society for Horticultural Science

VOLUME 32--1945

PUBLISHED BY THE SOCIETY MONTHLY
EXCEPT AUGUST AND SEPTEMBER

Publication Office: Free Press Printing Company, Burlington, Vermont
Subscription Price, \$3.00 per year

Entered as second-class matter at the post office at Burlington, Vt., May 4, 1939.

TABLE OF CONTENTS, VOLUME 32, 1945

No. 1, JANUARY

Plant growth under controlled conditions. IV. Response of California annuals to photoperiod and temperature HARLAN LEWIS AND F. W. WENT	1
Cultivation of excised stem tips of asparagus in vitro.....SHIH-WEI LOO	13
Vascularization of the vegetative shoots of <i>Helianthus</i> and <i>Sambucus</i>KATHERINE ESAU	18
Brazilian chytrids. V. <i>Nowakowskiella macrospora</i> n. sp., and other polycentric species.....JOHN S. KARLING	29
Growth and differentiation in the root tip of <i>Phleum pratense</i> RICHARD H. GOODWIN AND WILLIAM STEPKA	36
Controlling the pH of cultures of <i>Penicillium notatum</i> through its carbon and nitrogen nutrition ALBERT E. DIMOND AND GEORGE L. PELTIER	46

No. 2, FEBRUARY

The breeding of ornamental edible peaches for mild climates. I. Inheritance of tree and flower characters.....WALTER E. LAMMERTS	53
Apparent localization of <i>Fusarium</i> wilt resistance in the Pan American tomato.....P. H. HEINZE AND C. F. ANDRUS	62
Cleistogamy and chasmogamy in <i>Bromus carinatus</i> Hook. & Arn. JACK R. HARLAN	66
Periclinal chimeras in <i>Datura</i> in relation to the development and structure of the ovule.....SOPHIE SATINA	72
A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae.....WRAY M. BOWDEN	81
Desynapsis in the common wheat.....H. W. LI, W. K. PAO, AND C. H. LI	92

No. 3, MARCH

Studies on colchicine-induced autotetraploid barley. I and II. Cytological and morphological observations SHAO-LIN CHEN, SHU-MIN SHEN AND P. S. TANG	103
Growth stimulation by manganese sulphate, indole-3-acetic acid, and colchicine in the seed germination and early growth of several cultivated plants.....TSUNG-LE LOO AND YÜ-WEI TANG	106
Genetics of <i>Glomerella</i> . II. Fertilization between strains C. W. EDGERTON, S. J. P. CHILTON, AND G. B. LUCAS	115
Growth and vascular development in the shoot apex of <i>Sequoia sempervirens</i> (Lamb.) Endl. I. Structure and growth of the shoot apex.....CLARENCE STERLING	118
The calculation of tensions in <i>Cucurbita pepo</i>C. RALPH STOCKING	126
Anatomy of <i>Cryptostegia grandiflora</i> with special reference to the latex system.....H. WESTON BLASER	135
Natural breeding structure in the <i>Bromus carinatus</i> complex as determined by population analyses.....JACK R. HARLAN	142

The structure of the cell walls of <i>Aspergillus</i> and the theory of cellulose particles.....	EDWARD S. CASTLE	148
The cytoplasmic basis of intercellular patterns in vascular differentiation.....	EDMUND W. SINNOTT AND ROBERT BLOCH	151
The existence of physiological strains in <i>Physarum polycephalum</i>	WILLIAM D. GRAY	157
The production and characterization of ultraviolet-induced mutations in <i>Aspergillus terreus</i> . I. Production of the mutations.....	ALEXANDER HOLLAENDER, KENNETH B. RAPER, AND ROBERT D. COGHILL	160
The production and characterization of ultraviolet-induced mutations in <i>Aspergillus terreus</i> . II. Cultural and morphological characteristics of the mutations.....	KENNETH B. RAPER, ROBERT D. COGHILL, AND ALEXANDER HOLLAENDER	165

No. 4, APRIL)

Studies on colchicine-induced autotetraploid barley. III. Physiological studies.....	SHAO-LIN CHEN AND P. S. TANG	177
Studies on colchicine-induced autotetraploid barley. IV. Enzyme activities.....	SHAO-LIN CHEN AND P. S. TANG	180
Growth and phosphorus accumulation in cotton flowers as affected by meiosis and fertilization....	ORLIN BIDDULPH AND DONALD H. BROWN	182
Rapid total extraction of auxin from green plant tissue.....	G. S. AVERY, JR., J. BERGER AND R. O. WHITE	188
A list of chromosome numbers in higher plants. II. Menispermaceae to Verbenaceae.....	WRAY M. BOWDEN	191
Some additions to the genus <i>Dodonaea</i> L. (fam. Sapindaceae).....	EARL EDWARD SHERFF	202
The production and characterization of ultraviolet-induced mutations in <i>Aspergillus terreus</i> . III. Biochemical characteristics of the mutations.....	LEWIS B. LOCKWOOD, KENNETH B. RAPER, ANDREW J. MOYER AND ROBERT D. COGHILL	214
Quantitative irradiation experiments with <i>Neurospora crassa</i> . I. Experiments with X-rays.....	EVA R. SANSOME, M. DEMEREC AND ALEXANDER HOLLAENDER	218
Quantitative irradiation experiments with <i>Neurospora crassa</i> . II. Ultraviolet irradiation....	ALEXANDER HOLLAENDER, EVA R. SANSOME, E. ZIMMER AND M. DEMEREC	226

No. 5, MAY

Metastatic (graft) tumors of bacteria-free crown-galls on <i>Vinca rosea</i>	PHILIP R. WHITE	237
The extraction of auxin from tomato fruit.....	WESLEY P. JUDKINS	242
The anatomy of leaf abscission and experimental defoliation in guayule.....	FREDRICK T. ADDICOTT	250
The effect of indole-3-acetic acid on the dry weight of <i>Chlorella pyrenoidosa</i>	MELVIN AMOS BRANNON AND HAROLD MELVIN SELL	257
A morphologic study of the genus <i>Monoblepharella</i>	MARTHA E. SPRINGER	259

Auxin in <u>leaves</u> and its inhibitory effect on bud growth in guayule.....	PAUL F. SMITH	270
Observations on spiral grain in timber.....	J. H. PRIESTLEY	277
Rocky Mountain herbarium studies. VI.....	AVEN NELSON	284

No. 6, JUNE

The evolution of oxygen from suspensions of chloroplasts; the activity of various species and the effects of previous illumination of the leaves.....	JOHANNA KUMM AND C. S. FRENCH	291
Nutrient requirements in the germination of the conidia of <i>Glomerella cingulata</i>	CH'WAN-KWANG LIN	296
Some ecotypic relations of <i>Deschampsia caespitosa</i>	WILLIAM E. LAWRENCE	298
Plant nutrition in relation to disease development. I. Cabbage yellows.....	J. C. WALKER AND W. J. HOOKER	314
Factor Z ₂ and gametic reproduction by <i>Phycomyces</i> WILLIAM J. ROBBINS AND MARY BARTLEY SCHMITT		320
Endomitotic tapetal cell divisions in <i>Spinacia</i>	E. R. WITKUS	326
A remarkable tree-fall and an unusual type of graft-union failure ARTHUR J. EAMES AND L. G. COX		331
A physiological separation of two factors necessary for the formation of roots on cuttings....	J. VAN OVERBEEK AND LUIS E. GREGORY	336
New evidence on the telophase split in <i>Todea barbara</i>	IRENE MANTON	342

No. 7, JULY

The use of the C ¹³ isotope as a tracer for transport studies in plants.....	G. S. RABIDEAU AND G. O. BURR	349
✓ Growth in vitro of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar.....	ALBERT C. HILDEBRANDT, A. J. RIKER, AND B. M. DUGGAR	357
Brazilian chytrids. VI. <i>Rhopalophlyctis</i> and <i>Chytriomycetes</i> , two new chitinophyllic operculate genera.....	JOHN S. KARLING	362
Further studies on the chiasmata of the <i>Allium cepa</i> x <i>A. fistulosum</i> hybrid and its derivatives.....	S. L. EMSWELLER AND H. A. JONES	370
Growth and vascular development in the shoot apex of <i>Sequoia sempervirens</i> (Lamb.) Endl. II. Vascular development in relation to phyllotaxis.....	CLARENCE STERLING	380
The mechanism of colchicine-induced cytohistological changes in cranberry.....	HAIG DERMEN	387
Interspecific hybridization in <i>Parthenium</i> . I. Crosses between guayule (<i>P. argentatum</i>) and mariola (<i>P. incanum</i>)....	REED C. ROLLINS	395
Studies on <i>Chlorella vulgaris</i> . X. Influence of the age of the culture on the accumulation of chlorellin ROBERTSON PRATT, JOHN F. ONETO, AND JANE PRATT		405
A virus tumor disease of plants.....	L. M. BLACK	408
✓ An experimental analysis of alkaloid production in <i>Nicotiana</i> : The origin of nornicotine.....	RAY F. DAWSON	416

- Morphogenesis of fungus colonies in submerged shaken cultures
PAUL R. BURKHOLDER AND EDMUND W. SINNOTT 424
- A morphological, developmental, and cytological study of four
saprophytic chytrids. I. *Catenomyces persicinus* Hanson
ANNE M. HANSON 431
- The relation of growth to size in cucurbit fruits..... EDMUND W. SINNOTT 439

No. 8, OCTOBER

- New marine algae from southern California, III.....GEORGE J. HOLLENBERG 447
- Cytogenetics of certain *Triticum-Agropyron* hybrids and their
fertile derivatives.....R. MERTON LOVE AND C. A. SUNESON 451
- Origin and development of sclereids in the foliage leaf of
Trochodendron aralioides Sieb. & Zucc.....ADRIANCE S. .ER 456
- Plant growth under controlled conditions. V. The relation
between age, light, variety and thermoperiodicity of
tomatoes.....F. W. WENT 469
- A morphological, developmental, and cytological study of four
saprophytic chytrids. II. *Rhizophydium coronum* Hanson
ANNE MARIE HANSON 479
- Plant nutrition in relation to disease development. II. Cabbage
clubroot.....J. C. WALKER AND W. J. HOOKER 487
- Cell elongation and the development of root hairs in tomato
roots.....R. G. H. CORMACK 490
- Growth factor studies with *Spirodela polyrrhiza* (L.)
Schleid.....PAUL R. GORHAM 496
- Production of variable aneuploid numbers of chromosomes within
the root tips of *Paphiopedilum Wardii*.....ROBERT E. DUNCAN 506
- Growth factors for *Trichophyton mentagrophytes*
WILLIAM J. ROBBINS AND ROBERTA MA 509
- Effects of deficiencies of certain mineral elements on the develop-
ment of *Taraxacum kok-saghyz*.....BERNARD S. MEYER 523
- Influence of the proportions of KH_2PO_4 , MgSO_4 and NaNO_3
in the nutrient solution on the production of penicillin in
surface cultures.....ROBERTSON PRATT 528
- Habit of growth of *Rubus rosaefolius* Smith in Hawaii....CHARLES J. ENGARD 536

No. 9, NOVEMBER

- Silicon absorption by rye and sunflower.....ROBERT T. WHITTENBERGER 539
- Genetics of *Glomerella*. III. Crosses with a conidial strain
S. J. P. CHILTON, G. B. LUCAS, AND C. W. EDGERTON 549
- Evidence for genetic variation among apomictically produced plants
of several F_1 progenies of guayule (*Parthenium argentatum*)
and mariola (*P. incanum*).....REED C. ROLLINS 554
- A cytogenetic study of polyembryony in *Asparagus officinalis* L.
THOMAS E. RANDALL AND CHARLES M. RICK 560
- ✓ The effect of the moisture content of the soil upon the rate of
exudation.....J. JOSEPH McDERMOTT 570

Cell number in successive segments of <i>Avena</i> coleoptiles of different ages: Material for the biochemist G. S. AVERY, JR., MARGARET PIPER, AND PATRICIA SMITH	575
Brazilian chytrids. VII. Observations relative to sexuality in two new species of <i>Siphonaria</i>JOHN S. KARLING	580
Studies in the developmental anatomy of <i>Phlox Drummondii</i> Hook. I. The embryo.....HELENA A. MILLER AND RALPH H. WETMORE	588
Natural hybrids between <i>Oryzopsis hymenoides</i> and several species of <i>Stipa</i>B. LENNART JOHNSON	599
The known geographic distribution of the members of the Verbenaceae and Avicenniaceae.....HAROLD N. MOLDENKE	609

No. 10, DECEMBER

Accuracy of the local-lesion method for measuring virus activity. IV. Southern bean mosaic virus.....W. C. PRICE	613
Rubber in <i>Cryptostegia</i> leaf chlorenchyma ROBERT T. WHITTENBERGER AND ALBERT KELNER	619
Studies in the developmental anatomy of <i>Phlox Drummondii</i> Hook. II. The seedling:..HELENA A. MILLER AND RALPH H. WETMORE	628
The water factor in transplanting guayule.....LOUIS C. ERICKSON	634
Plant growth under controlled conditions. VI. Comparison between field and air-conditioned greenhouse culture of tomatoes.....F. W. WENT AND LLOYD COSPER	643
Flowering of Peruvian cube, <i>Lonchocarpus utilis</i> A. C. Smith, induced by girdling WILLIAM C. COOPER, ALBERT L. BURKETT AND ALEJANDRO HERR	655
The species concept in <i>Fusarium</i> with reference to Discolor and other sections.....WILLIAM C. SNYDER AND H. N. HANSEN	657
Auxin and nitrogen relationships in green plants GEORGE S. AVERY, JR., AND LOUISE POTTORF	666
Polyploidy, auxin and nitrogen in green plant tissue GEORGE S. AVERY, JR., AND LOUISE POTTORF	669
<i>Neurospora</i> I. Preliminary observations of the chromosomes of <i>Neurospora crassa</i>BARBARA MCCLINTOCK	671
<i>Neurospora</i> II. Methods of producing and detecting mutations concerned with nutritional requirements..G. W. BEADLE AND E. L. TATUM	678

PLANT GROWTH UNDER CONTROLLED CONDITIONS. IV. RESPONSE OF CALIFORNIA ANNUALS TO PHOTOPERIOD AND TEMPERATURE ¹

Harlan Lewis and F. W. Went

The role of the environment in producing morphological modifications of plants has long been a subject for speculation. In more recent years this problem has left the realm of speculation and has been approached experimentally, primarily by transplant experiments such as those conducted by Bonnier (1890, 1895, 1920), Hall (1932), Tureson (1922, 1925, 1930), and Clausen, Keck and Hiesey (1940). In all of these experiments the effect of different natural climates was investigated. But, because any natural environment is the sum total of numerous fluctuating and uncontrollable factors, which vary from year to year, the morphological modifications induced by varied environments cannot be attributed to any particular factor with any degree of certainty. In order to ascertain the specific factors responsible for given morphological modifications, it is, therefore, necessary to conduct experiments under controlled conditions. Such controlled conditions are now available in the air-conditioned greenhouses at the California Institute of Technology (Went, 1943) which were used for the experiment now described.

The most suitable plants to use for such an experiment seemed to be annuals because they com-

plete their life cycle within a few months, and hence differences in response between juvenile and mature plants can be observed in an experiment of fourteen weeks duration. Because it was our object to relate our experimental results to the natural ecology of the plants, local plants with whose distribution and ecology we were already somewhat familiar were employed. Preliminary experiments were conducted in 1941-42 using about eighty California annuals. From this group thirteen of the most suitable were selected for the present experiment. Seeds of all of these plants were supplied by the Rancho Santa Ana Botanic Garden, to whom the authors are accordingly indebted.

MATERIAL.—Following is a list of the annuals employed, with brief descriptions of their occurrence, and of the place of origin of the seed lots used.

Baeria chrysostoma T. & G. (Compositae).—Is abundant on plains, valleys and foothills at elevations below 2500 feet throughout California and southern Oregon. It flowers in March and April. The plants used in this experiment are of a strain originally collected at the lower end of Aliso Canyon, Rancho Santa Ana, San Bernardino County, California, by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 7780). Figures 6-8.

TABLE I.

Condition	1	2	3	4	5	6	7	8	9	10	11	12
Day temperature in degrees C. (8 a.m. to 4 p.m.)	19	19	19	19	19	19	19	19	19	19	26½	26½
Night temperature in degrees C. (4 p.m. to 8 a.m.)	19	19	19	19	19	7	26½	26½	13	7 ^b	13	26½
Photoperiod in hours ^a	8	12	14	18	24	8	8	10	10	10	10	10
<i>Baeria chrysostoma</i>	57	69	47	40	32	B	O	O	103	..	97	O
<i>Baileya multiradiata</i>	B	..	O	X
<i>Coreopsis californica</i>	B	B	77	54	43	B	B	..	B	..
<i>Eschscholtzia californica</i>	98	56
<i>Eschscholtzia Lobbii</i>	B	B	99	59	72	B	B	..	99
<i>Gilia tricolor</i>	80	B	79	80	..	80	87	B	89
<i>Godetia quadrivulnera</i>	B	97	85	..	O	X	O
<i>Linanthus montanus</i>	74	56	O	O
<i>Madia elegans</i>	76	..	B	81	64	..	O	O	O
<i>Mentzelia Lindleyi</i>	B	B	63	65	..	O	O	O
<i>Monardella lanceolata</i>	B	B	X
<i>Oenothera deltoides</i>
<i>Phacelia Parryi</i>	B	65	43	33	B	B	..	B	..

Numbers represent days required for first flower to bloom.

B = flower buds initiated at the end of the experiment (103 days).

O = plants dead.

X = plants visibly unhealthy.

^a The first 8 hours in conditions 1 through 7 are natural daylight; light for the additional hours was supplied by G.E. daylight fluorescent bulbs and Cooper-Hewitt lamps giving a combined intensity of 450 f.c. Conditions 8 through 12 were exposed to a natural length day which was approximately 10 hours during this experiment.

^b From 4 p.m. to 8 p.m. the temperature was 13°C.

[The Journal for December (31: 597-659) was issued January 12, 1945]
AMERICAN JOURNAL OF BOTANY, VOL. 32, NO. 1, JANUARY, 1945

Baileya multiradiata Harv. & Gray (Compositae)².—Occurs on the eastern Mojave Desert and eastward to Texas. It blooms for the most part from March through July. Strain collected on the Mojave Desert in the Clark Mountains 1.6 miles west of Pachalka Springs, San Bernardino County, California (elevation 4600 feet) by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 7000).

Coreopsis californica (Nutt.) Sharsmith (Compositae).—Occurs on the Mojave Desert eastward to Pima County, Arizona, on the western margin of the Colorado Desert, and in sandy valleys and mesas of the South Coast Ranges and cismontane Southern California southward to San Quintin, Baja California. It blooms in March, April and May. Strain originally collected on the Mojave Desert six miles south of Barstow on the road to Stoddard Well,

² Short lived perennial, but flowering as an annual.

San Bernardino County, California (elevation 2300 feet), by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 10281). Figure 12.

Eschscholtzia californica Cham. var. *crocea* Jepson (Papaveraceae)³.—Occurs on open grassy plains in the San Joaquin and Sacramento Valleys in California. It is said to have two seasonal flowering forms, a large orange-flowered vernal form and a small yellow-flowered aestival form (Jepson, 1922). Strain collected in the Sacramento Valley one mile north of Durham, Butte County, California (elevation 150 feet) by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 8787). Figure 12.

Eschscholtzia Lobbii Greene (Papaveraceae).—Occurs at elevations to 2000 feet on the open plains and foothills of the Sierra Nevada from Shasta to Kern Counties, California, and in the foothills of

³ Short lived perennial, but flowering as an annual.

TABLE 2.

	Flower initiation			Leaf size			Rate of leaf production			Withstand high (26½°C.) night temperature	Germination ^a		
	Photoperiod	Night temperature	Day temperature	Photoperiod	Night temperature	Day temperature	Photoperiod	Night temperature	Day temperature		Day 26½°C. Night 26½°C.	Day 26½°C. Night 19°C.	Day 26½°C. Night 19°C.
<i>Baeria chrysostoma</i>	+	19	O	+ ^b	+ ^b	19 ^c	O	+	O	no	no	yes	
<i>Baileya multiradiata</i>	+	O	O	O	+ ^b	O	O	+	O	no	
<i>Coreopsis californica</i>	+	O	O	+	+ ^d	19 ^c	O	+	O	no	no	yes	
<i>Eschscholtzia californica</i>	+	O	O	O	19	O	O	/	O	yes	yes	yes	
<i>Eschscholtzia Lobbii</i>	/	O	O	+	+ ^d	O	O	+	26½ ^e	yes	yes	yes	
<i>Gilia tricolor</i>	/	26½ ^e	O	O	+ ^d	O	O	+	O	yes	
<i>Godetia quadrivulnera</i>	+	O	O	O	O	O	O ^g	+	O	no	yes	yes	
<i>Linanthus montanus</i>	+	O	O	O	O	O	O	+	O	no	no	yes	
<i>Madia elegans</i>	B	19 ^h	O	O	O ⁱ	19 ^c	O	+	O	no	no	yes	
<i>Mentzelia Lindleyi</i>	+	19 ^j	O	O	+ ^d	O	O	/	O	no	no	yes	
<i>Monardella lanceolata</i>	+	O	O	+ ^k	O ⁱ	O	/	+	O	yes ^m	yes	yes	
<i>Oenothera deltoides</i>	O	O	O	O	O ⁱ	O	O	+	O	yes	yes	yes	
<i>Phacelia Parryi</i>	+	19 ^j	19	+ ^b	+ ^d	19	O	+	O	yes	yes	yes	

O = no response independent of factor under consideration.

+

/ = probable positive correlation.

B = bimodal response.

19 or 26½ = optimal temperature.

+ = special case, see superscript ^d.

^a From preliminary experiments.

^b Initially correlated, that is, until modified by flower initiation, adverse effects of high night temperature, etc.

^c With a 13° night and natural length day.

^d Largest leaves produced at 13° and the smallest at 7° or 26½° with a 19° natural length day, but with a 19° 8-hour photoperiod the largest leaves are produced at 7°.

^e With a 26° night and natural length day; with a 13° night and natural length day, response is independent of day temperature.

^f Flowers very small.

^g With an 8-hour photoperiod, the rate is somewhat lower than with longer photoperiods.

^h With a 19° 8-hour photoperiod, only; there is no effect with a natural length day.

ⁱ Leaves produced with a 13° night are somewhat larger than those produced at other temperatures.

^j With a 19° natural length day.

^k A threshold is reached with 14 hours of light (8 natural daylight, 6 artificial); beyond this point there is no increase in leaf size with additional light.

^l Leaf size is notably reduced with a 7° night and 19° natural length day.

^m Plants grown at 26° day and night were visibly unhealthy at the end of the experiment.

the North Coast Ranges bordering the Sacramento Valley. It blooms in March and April. Strain originally collected near Clovis, Fresno County, California (elevation 400 feet) by A. L. Chickering.

Gilia tricolor Benth. (Polemoniaceae).—Occurs on grassy slopes and hillsides up to 3500 feet in the Sacramento Valley, on the western margin of the San Joaquin Valley, and in the Coast Ranges from Santa Clara County north to Humboldt County, California. It blooms in March, April and May. Strain originally collected in Tejon Canyon, Kern County, California (elevation 2300 feet) by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 6741). Figure 12.

Godetia quadrivulnera (Dougl.) Spach ssp. *typica* C. L. Hitchc. (Onagraceae).—Occurs at elevations below 4000 feet on dry hillsides throughout California (except on the deserts) north to Oregon and Washington. It blooms from late April through July.

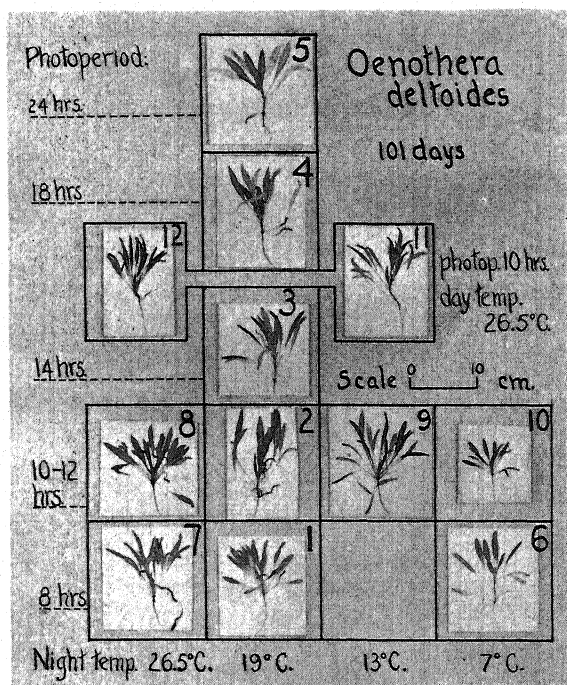


Fig. 1. *Oenothera deltoidea* var. *cognata*. Photographs of herbarium specimens collected 101 days after sowing. Only one representative plant per treatment shown. Numbers in upper right hand corner of each square correspond to condition numbers in table 1. Squares arranged according to night temperature from right to left. During day all plants were subjected to 19°C. except conditions 11 and 12, which were maintained at 26.5°C. In vertical order the conditions are arranged according to the length of photoperiod, except conditions 11 and 12, which had the same photoperiod as conditions 8, 9, and 10 (natural daylight).

Strain originally collected one-fourth mile southwest of Muddy Springs near Roundtop Peak, Rancho Santa Ana, Orange County, California (ele-

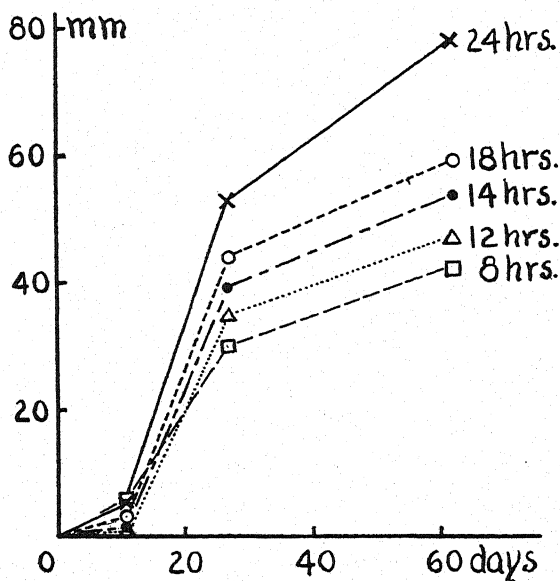
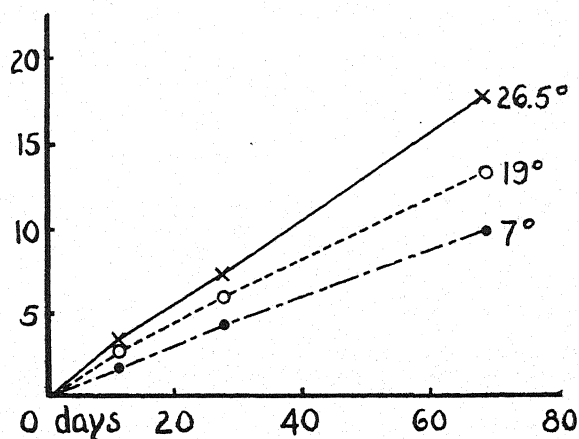


Fig. 2-3.—Fig. 2 (above). Leaf production of *Monardella lanceolata* under different night temperatures. For all plants: day temperature 19°C., photoperiod 8 hours.—Fig. 3 (below). Leaf size of *Eschscholtzia Lobbii* as a function of age and photoperiod. All plants grown at a constant temperature of 19°C. day and night.

vation 1400 feet) by E. R. Johnson (Rancho Santa Ana Botanic Garden Herbarium No. 4996).

Linanthus montanus Greene (Polemoniaceae).—Occurs at elevations of 1000 to 5000 feet in the oak woodland of the Sierra Nevada from Calaveras County to Tulare County, California. It blooms in April, May and June. Strain collected 2.8 miles above Dunlap on the Sand Creek cut-off to Miramonte, Fresno County, California (elevation 2500 feet) by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 5061). Figure 12.

Madia elegans Don var. *densifolia* Jepson (Compositae).—Occurs on dry hillsides at elevations below 3500 feet in the Sierra Nevada foothills from Fresno County, California, north to Oregon and in the Coast Ranges from San Luis Obispo County north to Mendocino County, California. It blooms for the most part from June through September. Strain collected one-half mile south of Mokelumne Hill on the road to San Andreas, Calaveras County, California (elevation 1500 feet), by C. B. Wolf. (Rancho Santa Ana Botanic Garden Herbarium No. 5204).

Mentzelia Lindleyi T. & G. ssp. *typica* Wolf (Loasaceae).—Grows at elevations of 1500 to 4000 feet in the South Coast Ranges from Alameda County, south to Monterey and San Benito Counties, California. It blooms in April and May. Strain obtained from England, by Theodore Payne. The strain is presumably derived from seeds collected in California by David Douglas in 1830-32.

Monardella lanceolata Gray (Labiateae).—Occurs in the foothills and mountains at an elevation of 1000 to 6000 feet (usually in the yellow pine forest) in the Sierra Nevada from Shasta County south to Tulare County, California, in the Coast Ranges in Santa Barbara and Ventura Counties, in the San Gabriel and San Bernardino Mountains south to San Diego County. It blooms for the most part in June, July, and August. Strain originally collected

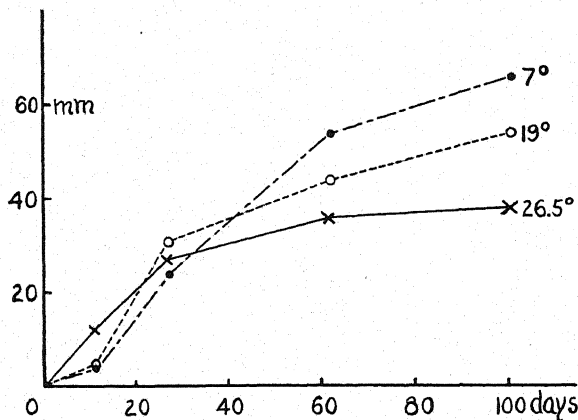


Fig. 4. Leaf size of *Eschscholtzia Lobbii* as a function of time and night temperature. Day temperature 19°C, photoperiod 8 hours.

at Bradshaw's Camp on the Bass Lake to Wawona road, three miles from the Mariposa County Line in Madera County, California (elevation 3500 feet), by C. B. Wolf (Rancho Santa Ana Botanic Garden Herbarium No. 5195).

Oenothera deltoidea Torr. & Frem. var. *cognata* (Jepson) Munz (Onagraceae).—Occurs in sandy places in the San Joaquin Valley and western part of the Mojave Desert at elevations below 2500 feet. It flowers for the most part in March, April and May. Strain collected on the north side of the Merced River on U. S. Highway 99 between Merced

and Modesto, Merced County, California (elevation 100 feet), by C. B. Wolf. (Rancho Santa Ana Botanic Garden Herbarium No. 5162).

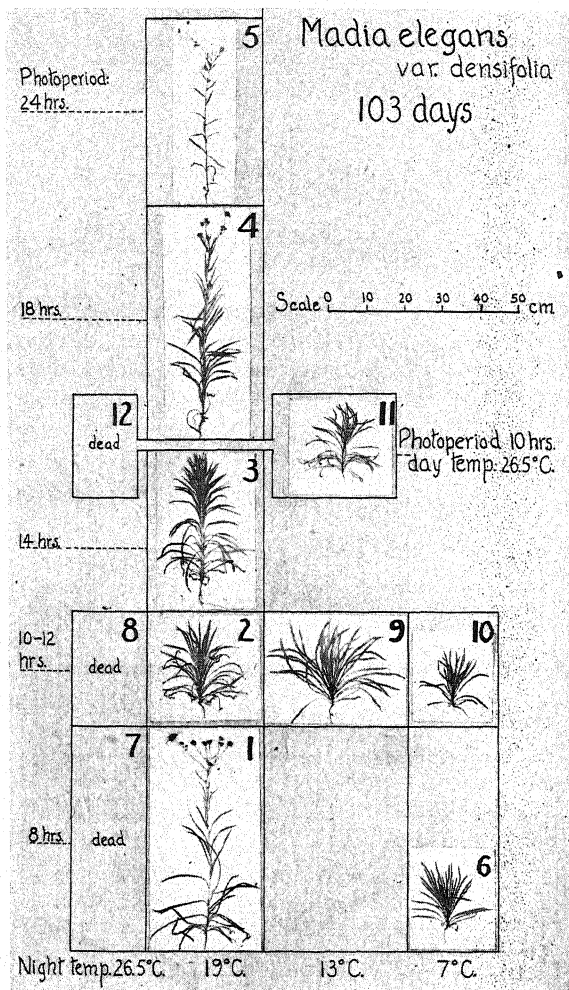


Fig. 5. Development of *Madia elegans* var. *densifolia*, 103 days after sowing. Only representative plant per treatment shown. For further explanation, see figure 1.

Phacelia Parryi Torr. (Hydrophyllaceae).—Occurs in burns and open places in the chaparral at elevations below 2500 feet in the Coast Ranges from the Santa Lucia Mountains southward to Baja California. It flowers from March through May. Strain grown for many years by Theodore Payne. Figures 9-11.

METHODS.—The experiment was begun October 5, 1943, and was continued for 103 days until January 12, 1944. The seeds were sown in 2-inch square plant bands in a uniform mixture of equal parts of sand and fine leaf mold. The plant bands were arranged in redwood flats, and to each species was allotted five bands per flat. A total of twelve flats was thus arranged, all identical and each containing the seeds of thirteen species. All of the seeds were

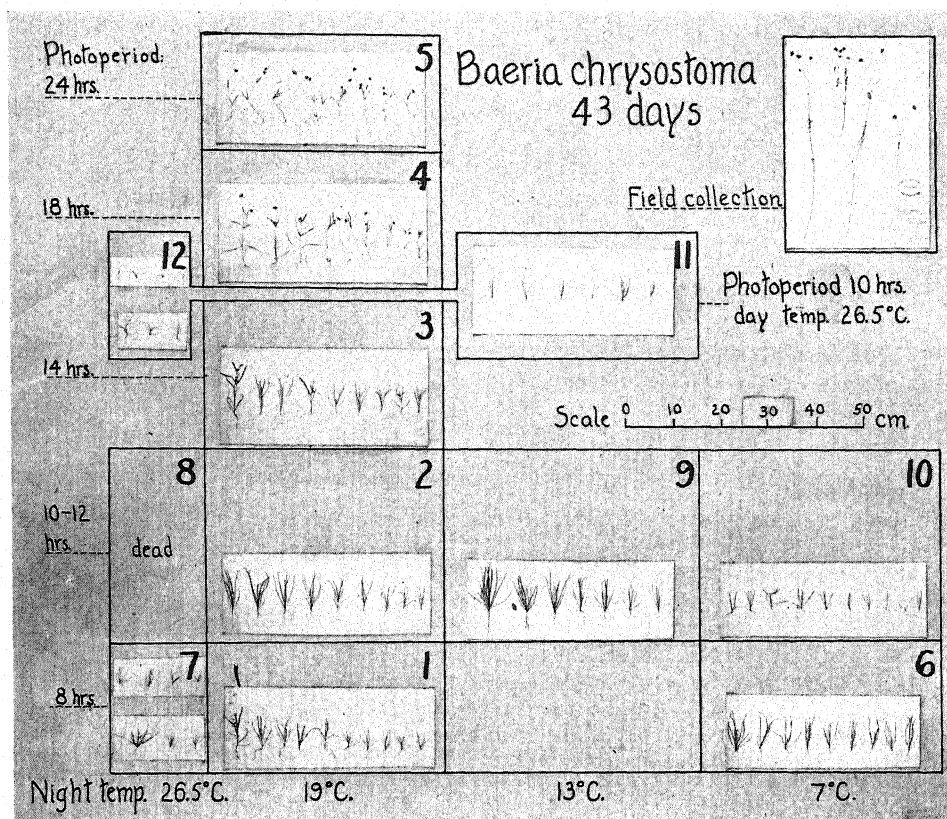


Fig. 6. Development of *Baeria chrysostoma*, 43 days after germination. For each condition, 6-10 plants are shown, representing the range of variability. Photo in upper right hand corner shows the original plants, from which the seed stock used in this experiment was collected. For further explanation, see figure 1.

germinated under identical conditions in an ordinary greenhouse. After germination and while the plants were still in the cotyledon stage they were thinned to seven per plant band. Thereupon, approximately three days after germination, the flats were subjected to the twelve conditions listed in table 1. Hence, at the beginning of the experiment 35 individuals of thirteen species were subjected to each of twelve artificial environments varied with respect to photoperiod and temperature. The humidity under all conditions was approximately 70 per cent. During the day (8 a.m. to 4 p.m.) all plants were subjected to natural daylight in the greenhouses where the intensity is approximately 60 per cent that of outside daylight. The intensity of the supplementary light during night was approximately 450 f.c. The plants were kept weeded and were thinned from time to time to prevent crowding and to insure that all plants were of the same age. At the end of the experiment there were three plants in each band except in the case of some of the larger species which were in some instances thinned to but a single plant per band. The plants were watered one day a week with Hoagland's nutrient solution, on other days with tap water. The seedlings were sprayed with a solution of Semesan to prevent damping off.

Aphids and other pests were combatted by regular spraying with greenhouse Volck.

Records for two purposes were made at intervals throughout the experiment. First, measurements were made as an index to (1) the amount of growth in length of the longest leaf and, (2) the number of leaves produced on the main stem. Second, the date of the opening of the first flower of each species under the various conditions was recorded as a measure of photoperiodicity. At the time of measurement, about sixty plants of each species (five from each condition) were harvested and pressed for future reference. These specimens were selected to represent the range of variation within each condition. They have been deposited with the herbarium of the University of California, Los Angeles.

RESULTS.—*Oenothera deltoidea cognata* (fig. 1) showed an exceptional degree of uniformity in response to all of the conditions (except condition 10 in which the plants were considerably smaller). However, throughout this experiment none of the individuals matured, but all remained in the rosette stage, a fact which is not wholly surprising since this species normally remains in this stage until the season is well advanced. Had the experiment continued until the flowering stage was reached, this

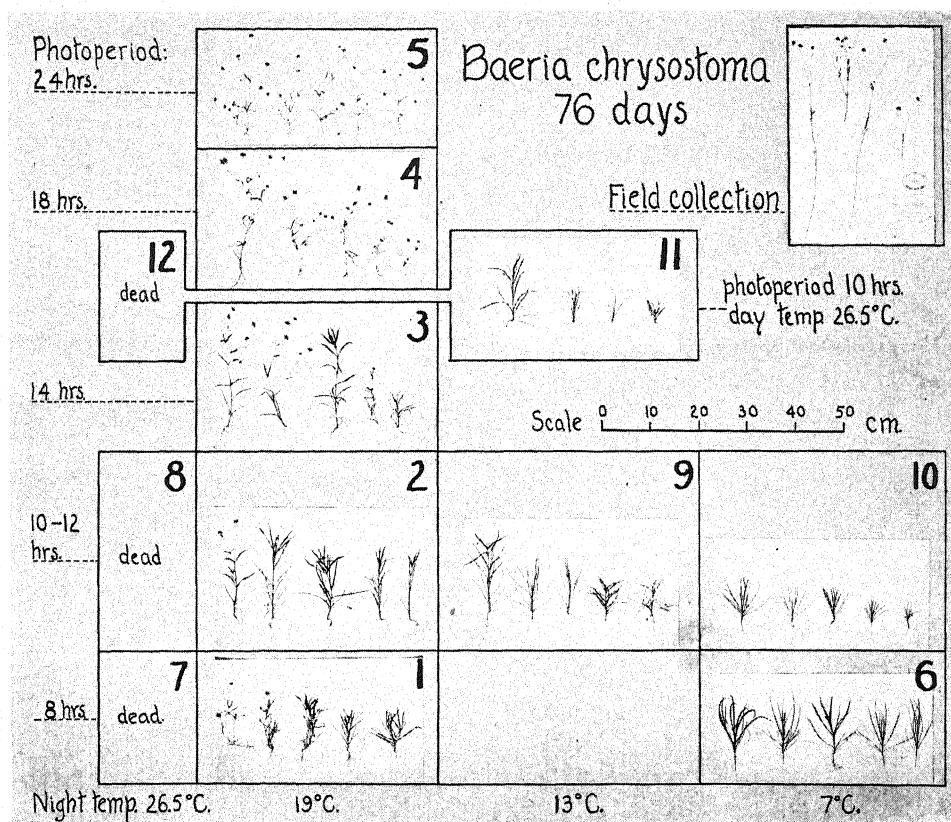


Fig. 7. Same as figure 6, but plants harvested 76 days after sowing.

species might also have shown photoperiodicity and thermoperiodicity. The principal difference induced by the different conditions was that the rate of leaf production was found to be directly correlated with the night temperature (table 2), but the difference between the highest rate and the lowest rate was insufficient to produce a visible difference in the photographs.

Godetia quadrivulnera was a long-day plant with respect to flowering (table 2). Leaf size was uniform under all conditions. The rate of leaf production was directly correlated with night temperature (as in fig. 2).

Linanthus montanus (fig. 12) was almost identical with *Godetia quadrivulnera* in its response.

Baileya multiradiata responded essentially like *Godetia quadrivulnera* save with respect to leaf size (table 2), in which one finds a direct correlation with the night temperature until the adverse effects of high night temperature become effective.

Eschscholtzia californica crocea (fig. 12) had a pattern of response similar to that of *Baileya multiradiata*. The rate of leaf production was probably correlated with the night temperature, because, by and large, more leaves were produced at the higher night temperatures.

Eschscholtzia Lobbii differed in response from *E. californica* principally in the direct response of

leaf size to the length of the photoperiod (fig. 3) and in the preference of a lower night temperature for the development of largest leaves (fig. 4).

Coreopsis californica (fig. 12) responded in essentially the same way as *Eschscholtzia Lobbii*.

Gilia tricolor (fig. 12) was photoperiodic, although flowering was not directly correlated with the length of the photoperiod. Occasional precocious blooming in the shorter photoperiods was exceptional. Flower initiation may also be stimulated by warm night temperatures but the flowers produced under such conditions were very much smaller than those produced at cooler temperatures. These small flowers were very similar to and recalled the flowers of Polemoniaceae often found on stunted plants growing in nature under "adverse" conditions.

Mentzelia Lindleyi was almost identical with *Gilia tricolor* in its response. It differed in its lack of direct correlation of the rate of leaf production with night temperature (table 2). This may in part have been due to an inadequate sample. However, the data strongly suggest that, except for the higher temperatures, the rate of leaf production of this species may be but little affected by night temperature.

Monardella lanceolata was unique among this group of plants in showing a positive correlation

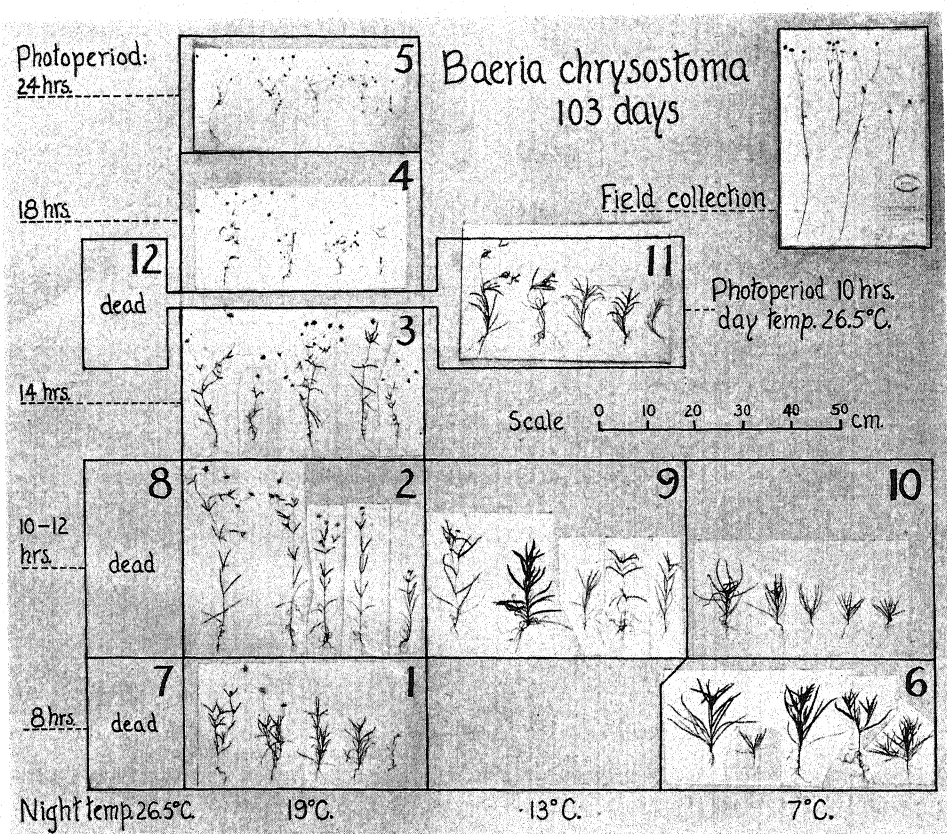


Fig. 8. Same as figures 6 and 7; plants harvested 103 days after sowing.

between the length of the photoperiod and the rate of leaf production. The differences in rate of leaf production thus produced were not nearly so great, however, as those produced in this species by the various night temperatures (fig. 2). Leaf size showed a marked response to an increase in photoperiod up to 14 hours of light (conditions, 1, 2, and 3), but with a 14-hour exposure a threshold was reached beyond which additional light had no further effect upon leaf size.

Madia elegans densifolia was unique in its photoperiodic response with respect to flowering (fig. 5). The response definitely followed a bimodal curve with one optimum near eight hours of daylight and a second optimum with 18 to 24 hours of light (eight hours natural daylight plus 10-16 hours of artificial light). Flower initiation was very definitely retarded in the intermediate photoperiods. That this phenomenon is not the result of an error in experimentation is demonstrated by the fact that the other photoperiodic plants, for example, *Phacelia Parryi* (fig. 11), although growing in the same flat, did not initiate flower buds under the eight-hour photoperiod, and that already after 79 days 80 per cent of the *Madia* plants in that condition flowered or had large flower buds.

Baeria chrysostoma was already known to be photoperiodic with respect to flower initiation (Sivori and Went, 1944). In the experiments by these authors *Baeria* was found to require a minimum photoperiod of fifteen hours of artificial light for flowering. The present experiment indicates that *Baeria* will flower with a photoperiod as short as eight hours of daylight (fig. 8), provided that it is given sufficient time (in this case 57 days). *Baeria*, therefore, presents the case of a plant in which the photoperiodic response changes with age; when less than thirty days old, it is a long day plant, but later it seems to become photoperiodically indifferent. It is of some interest to note that the optimum conditions can readily be modified by the duration of the experiment. Had the present experiment been brought to a close at the end of 43 days (fig. 6), it is quite obvious that the best developed plants would have been found in condition 5. However, those plants which were a bit slower to develop because of a shorter photoperiod eventually exceeded those which were first to mature, for after 76 days (fig. 7) the plants grown under condition 4 were best developed. After 103 days these in turn were surpassed by the plants grown under condition 3 (fig. 8).

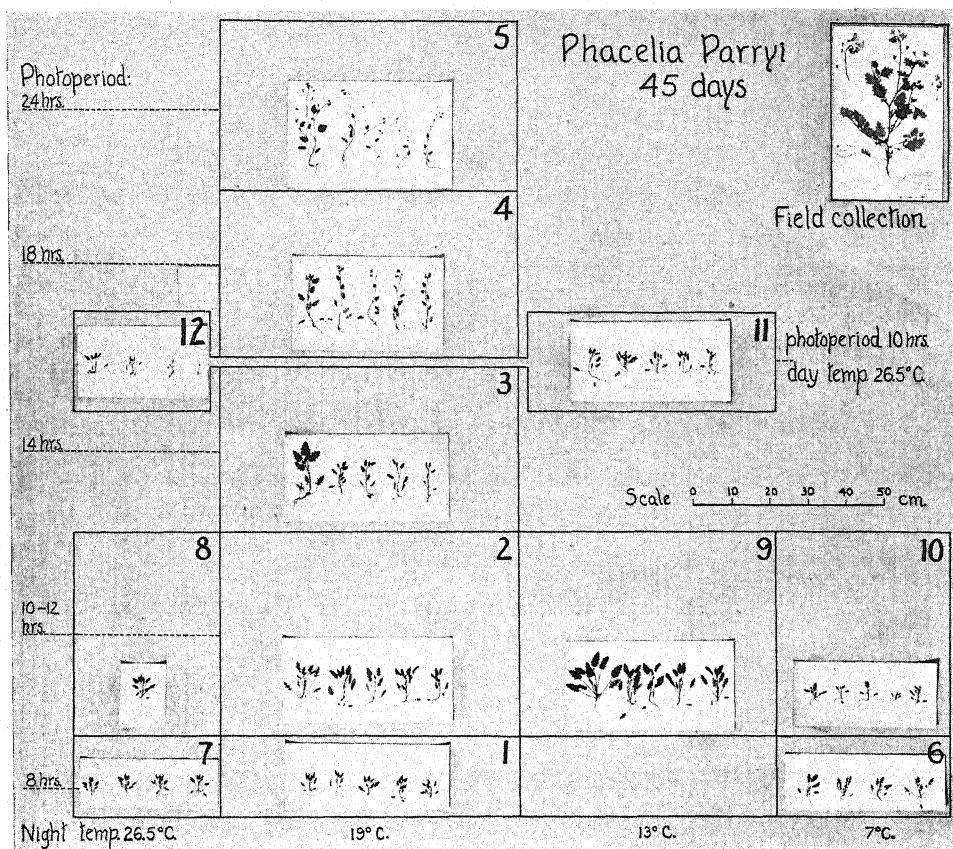


Fig. 9. Development of *Phacelia Parryi*, 45 days after sowing. For explanation see figures 1 and 6.

Phacelia Parryi was also shown by Sivori and Went (1944) to be photoperiodic with respect to flowering. In this experiment it closely paralleled *Baeria chrysostoma*. However, the change in photoperiodic response with age was less pronounced in *Phacelia Parryi*, for at the end of the experiment there was no trace of flower initiation in the plants grown with but eight hours of daylight (fig. 11). The conditions for flower initiation may not necessarily be the same as those for flowering in this species, since in the twelve-hour photoperiod many flower buds are initiated in which only the calyx matures and the remainder of the flower aborts. There was no indication from the present experiment as to the cause of this abortion. However, aborted flowers were no more frequent on our plants than on most plants of this species found in nature. Here, as with *Baeria*, the apparent optimum development is conditioned by the length of the experiment (figs. 9, 10, and 11). Attention should be called to the striking difference in the shape of the leaves, and particularly in the leaf margins of the plants grown, for example, under conditions 5 and 3 (fig. 11). The former have small, once lobed or dentate leaves while those in condition 3 are much larger, rounder, and with doubly crenate margins.

DISCUSSION AND CONCLUSIONS.—*Effect of photoperiod.*—Reference to tables 1 and 2 demonstrates that the most striking effect of photoperiod is its effect upon flower initiation. All but two of these spring-flowering annuals are long day plants. In some cases there is also an increase in the size of the leaves with an increase in length of photoperiod. Since these annuals do not store appreciable amounts of carbohydrates, and since the intensity of the supplementary light during night was fairly high, it is likely that in some cases the length of the photoperiod became the limiting factor in the growth of the leaves. If food is the limiting factor, one might expect an increase in leaf size corresponding to the length of day. With two possible exceptions (*Monardella* and *Godetia*) the length of the photoperiod has but little to do with the rate of leaf production. From figures 8 and 11 it also follows that the photoperiodic behavior depends largely upon night temperature.

Effect of temperature.—The day temperatures employed in this experiment seldom produce any differences of response, whether of flower initiation, size of leaves or rate of leaf production. Night temperature, however, has, in general, a pronounced effect upon the rate of leaf production and also upon leaf size. The higher the night temperature,

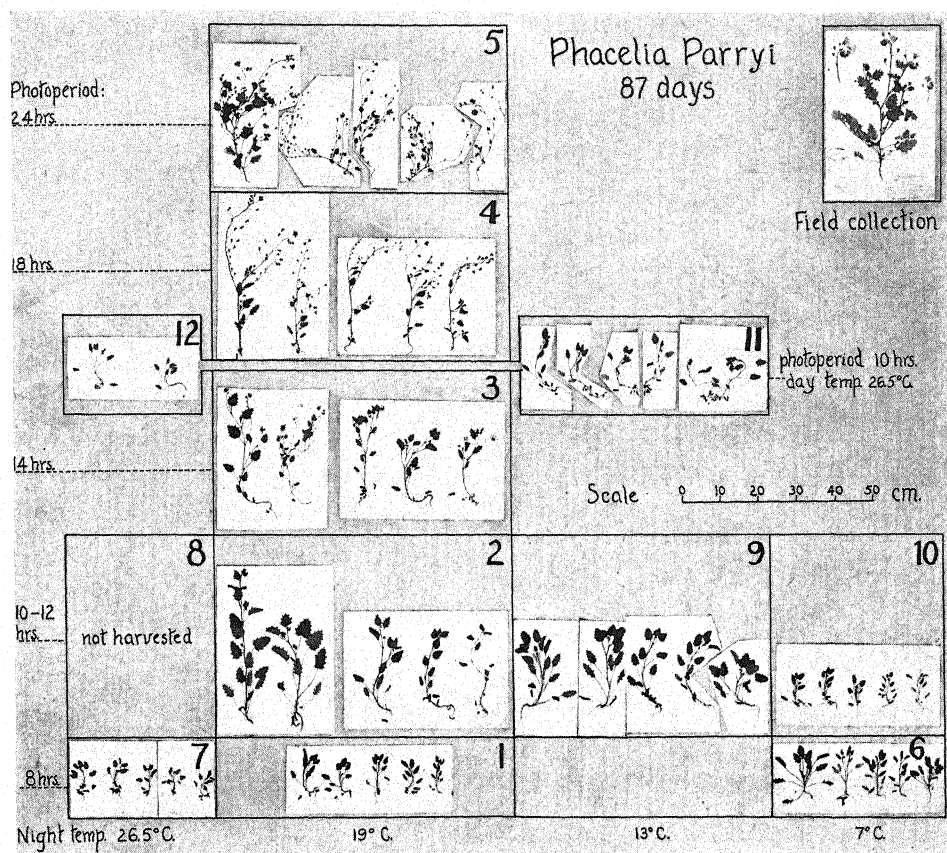


Fig. 10. Same as figure 9; plants harvested after 87 days.

the greater the rate of leaf production, a rate which is constant throughout the vegetative development of the plant so long as the night temperature remains constant and the plant remains healthy (fig. 2). This phenomenon was observed in most of the other plants observed. The constant rate of leaf production is a direct expression of a constancy in the development of the apical meristem. Since there are approximately the same number of cell divisions between the initiation of successive primordia, it follows that the effect of night temperature on the rate of leaf production must be the result of a fairly high temperature coefficient of the rate of cell division during night. Such a temperature effect on cell division was found, e.g., by Laughlin (1919) in *Allium Cepa* root tips.

Night temperature also effects leaf size. During the first two weeks, ten out of the thirteen species produced their largest leaves under conditions of a high ($26\frac{1}{2}^{\circ}\text{C}.$) night temperature. However, in no case did this condition continue. On the contrary, as the plants matured, the largest leaves were produced under cooler night temperatures (as in fig. 4). This shift from higher to lower optimal night temperatures, when the plants have passed

the seedling stage, is similar to that found in tomatoes (Went, 1944).

As can be seen from tables 1 and 2, approximately half of the species with which we are concerned were killed by high ($26\frac{1}{2}^{\circ}\text{C}.$) night temperature. Even though the others survived, many individuals tended to be weak and prostrate. In no case was a day temperature of $26\frac{1}{2}^{\circ}\text{C}.$ lethal unless associated with a $26\frac{1}{2}^{\circ}\text{C}.$ night temperature. It is most interesting to note that with one exception, seeds of the plants killed by high night temperature were unable to germinate at this night temperature (table 2). This indicates that the same factors responsible for the death of the plants at high night temperature are also operative in the seedlings or embryos.

Morphological modifications.—These fall into two classes, namely, differences in size, and differences in aspect or habit. The modifications of size, whether of leaf, stem or flower, are not of the sort that would ordinarily influence a taxonomist, with the exception of *Gilia tricolor*, a representative of the Polemoniaceae. In this case, the flowers produced under a high ($26\frac{1}{2}^{\circ}\text{C}.$) night temperature are much smaller than those produced at lower

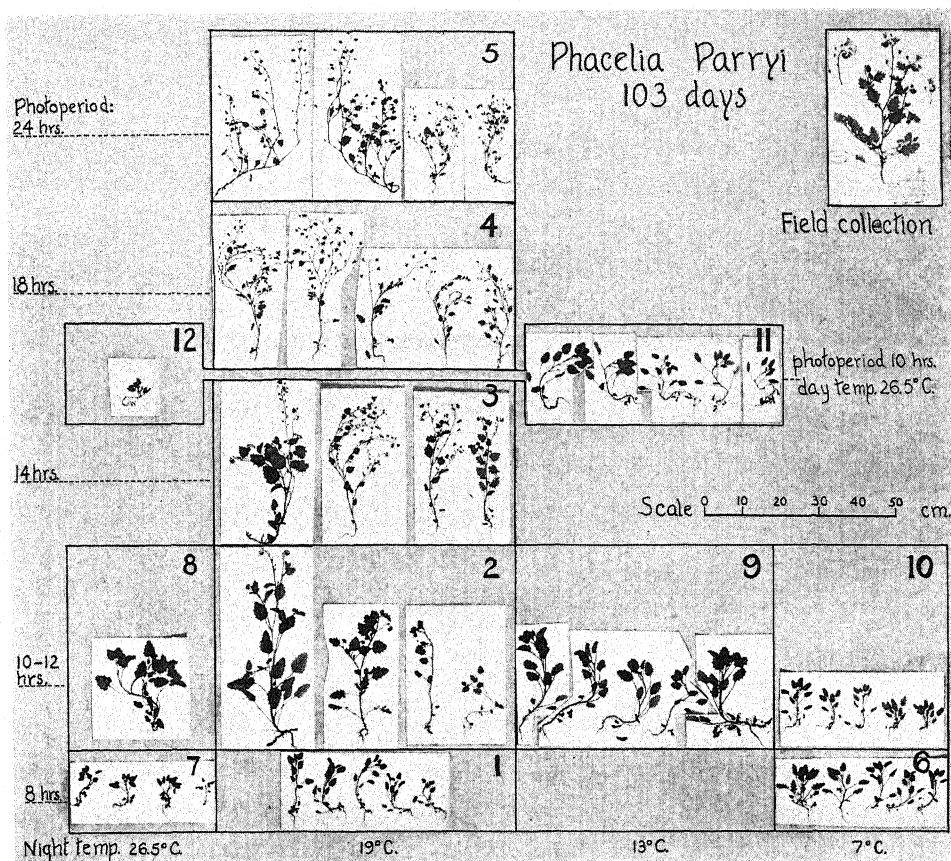


Fig. 11. Same as figures 9 and 10; plants harvested after 103 days.

night temperatures. As mentioned above, this is not surprising as one frequently finds an astonishing range in flower size on this and other species of the same or related genera. Our observations suggest that these differences may not be wholly genetic but may be in part modifications. The conformation and relative proportions of the flower, however, were not changed. This indicates that, as a rule, proportional measurements are more suitable in delimiting closely allied entities than absolute measurements, a practice long followed by taxonomists.

The most conspicuous differences in habit observed in this experiment are due to differences in the number of cauline leaves. This number is a function of the time of flower initiation which in our plants is influenced primarily by the length of the photoperiod. We have already mentioned the striking differences in the leaf margins of *Phacelia Parryi* when grown with short and long photoperiods. The juvenile leaves of this species, produced at the first few nodes, are once-lobed or dentate, those formed later have doubly crenate margins, while those in the inflorescence are again once-lobed or dentate. The lack of leaves with doubly crenate margins on the plants subjected to long photoperiods is due to the early initiation of

flower buds after only four or five nodes have been formed. Hence, although mature in the sense of producing flowers, such plants possess only leaves of one type. Whatever the species, comparison of leaves produced at a given node showed no differences in proportion, venation or margins beyond those to be expected in a random sample from a natural population. Our plants, grown under controlled conditions, are comparable in size and habit to plants of the same strain as it was found in nature or grown in the field (fig. 12).

Ecological correlations.—It might be expected that one would be able to correlate the response of these plants under controlled conditions with their responses in nature and to find some of the factors effective in limiting their distribution. But this particular experiment has yielded no such information. The reasons are perhaps several. It is quite evident that the limits used in this experiment were not sufficiently wide and that a greater range of artificial environments was needed. Then, too, other factors such as water relations may be of more importance than light and temperature. It may also be that this material was not suitable for showing differences in adaptation, for it is quite possible that these annuals all represent more or less the same ecological type. The life cycle of these an-

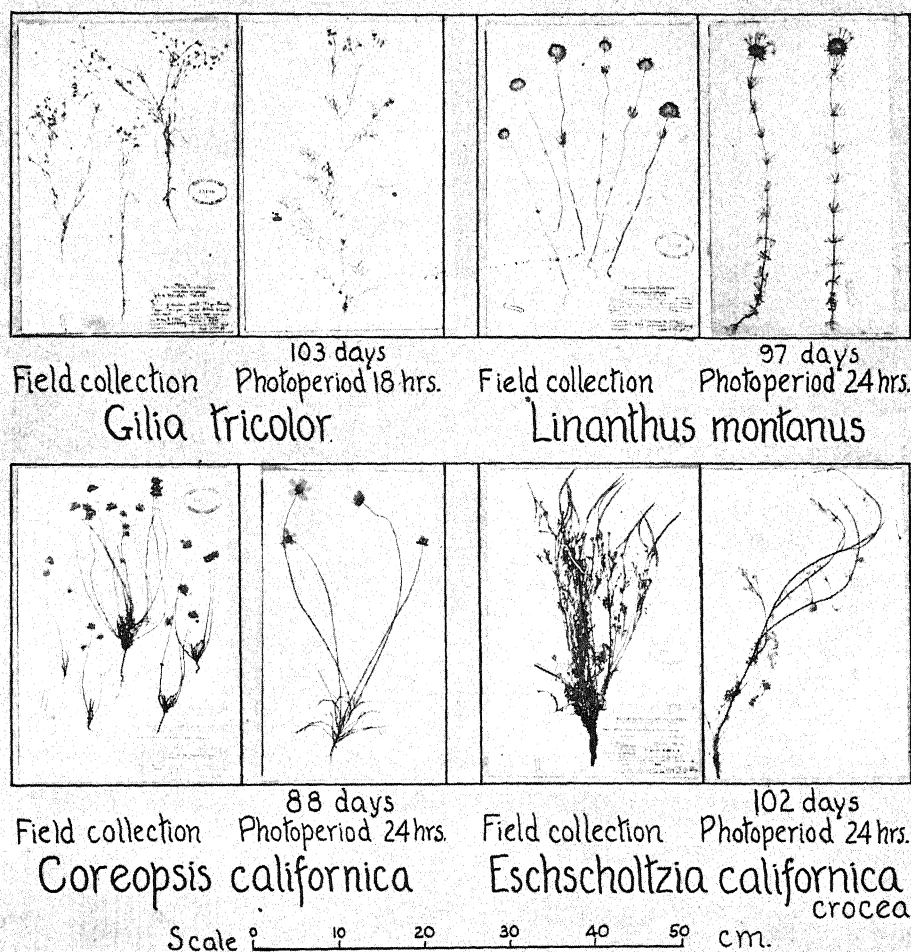


Fig. 12. Comparison of the original collection of some of the plants used in the present experiment (left photo of each pair) with some of the experimental plants which had a comparable development (right hand photos). They all came from condition 5, except *Coreopsis californica*, which came from condition 4 (c.f. table 1).

nuals is short and they spend most of the year in a dormant stage as seeds, hence they may grow in diverse climatic regions, so long as the conditions are suitable at some time during the year for them to germinate and complete their short cycle. Therefore, it does not follow that two annuals which grow in different climatic regions necessarily represent different ecological types, for they may actually grow under a regime which both regions have in common. To analyze the factors which limit the distribution of a species, it would be necessary to use a series of samples from different regions rather than a single sample, as in the case of this experiment, and it would be necessary to have a complete set of phenological and meteorological data concerning the races under investigation.

A species usually has a broad range of potential form. When the conditions under which a given biotype grows in nature are fairly uniform during successive growing seasons, considerable uniformity

among the individuals can be expected. If, on the other hand, such a biotype grows under a wide range of climatic, edaphic and biotic conditions, great variability is to be expected. Since all of the modifications that are included in the range of this variability belong to the same species, it follows that any concept of a species should include the response of its components to all potential growing conditions at all stages of their development. Such a concept would thus include all of the responses of which a genotype is capable and would make it possible to define, within limits, the conditions under which a specimen of any given biotype has grown. An initial contribution to such a concept of the species *Baeria chrysostoma* is shown in figures 6 to 8. The response in time of *Phacelia Parryi* to various temperatures and photoperiods is shown in figures 9 to 11. It can be seen that the specimens collected in nature or under natural growing conditions can easily be matched by specimens grow-

ing under controlled conditions. For every species investigated in detail by methods like those described it may thus become possible to identify the growing conditions in nature by the size, habit and condition of the plant. A depauperate specimen does not necessarily mean that it grew under nutrient or water deficiency; on the contrary, some of the smallest *Baeria* plants (condition 5) grew under such conditions that it developed, flowered and set seed in the shortest time. It was observed in the Sierra Nevada that *Linanthus montanus* when growing on the normal slope of the mountain had uniformly 4-5 nodes below the inflorescence while those on an exposed rocky outcropping in the same area, although in the same stage of flowering, had about eight nodes. This difference may well be attributed to earlier germination on the outcropping as a result of the earlier disappearance of snow on the site. All plants flowered at the same time due to the pronounced photoperiodicity of *Linanthus montanus* (table 1). Such observations strongly suggest the feasibility of correlation of field observations with experimental data. To show how well the plants grew under controlled conditions, figure 12 combines pictures of the original collection of the plant in the wild and the best specimens grown under the described conditions.

SUMMARY

Thirteen species of California annuals were grown under twelve different controlled conditions of temperature and photoperiod in air-conditioned greenhouses. All but two of these annuals proved to be long day plants. The photoperiodic response

of one, *Madia elegans*, follows a unique bimodal curve. In several cases the size of the leaves is directly correlated with the length of the photoperiod.

Day temperatures seldom produce any differences in response, whether of flower initiation, size of leaves or rate of leaf production. Night temperature, on the other hand, has, in general, a pronounced effect upon the rate of leaf production and also upon leaf size. The rate of leaf production remains constant throughout the vegetative development of the plant so long as the night temperature remains constant. There is, in general, a shift in optimal conditions for the production of largest leaves.

The morphological modifications are of two kinds, namely, differences in size and differences in habit. The differences in habit are attributable to differences in time of flower initiation. Comparison of leaves produced at a given node showed no differences in proportion, pubescence, venation or margin beyond those to be expected in a random sample from a natural population. The modifications described are not of the kind or magnitude which would ordinarily influence a taxonomist.

No correlation could be made between the responses of these plants under controlled conditions with their responses in nature but the methods used should be applicable to studies of plant distribution and the nature of species.

WILLIAM G. KERCKHOFF LABORATORIES,
CALIFORNIA INSTITUTE OF TECHNOLOGY,
PASADENA, CALIFORNIA

LITERATURE CITED

- BONNIER, G. 1890. Cultures expérimentales dans les Alpes et dans les Pyrénées. Rev. gén. de Botanique 2: 513-546.
- . 1895. Recherches expérimentales sur l'adaptation des plantes au climat alpin. Ann. Sci. Nat., Bot. 7th ser., 20: 217-358.
- . 1920. Nouvelles observations sur les cultures expérimentales à diverses altitudes. Rev. gén. de Botanique 32: 305-326.
- CLAUSEN, J., D. D. KECK, AND WM. M. HIESEY. 1940. Experimental studies on the nature of species. I. Effects of varied environments on western North American Plants. Carnegie Inst. Washington Publ. No. 520. 452 pp.
- HALL, H. M. 1932. Heredity and environment—as illustrated by transplant experiments. Sci. Monthly 35: 289-302.
- JEPSON, W. L. 1922. A flora of California I: 7 pp. 564-570. Berkeley. Univ. of Calif. Assoc. Students Store.
- LAUGHLIN, H. H. 1919. Duration of the several mitotic stages in the dividing root-tip cells of the common onion. Carnegie Inst. Washington Publ. No. 265. 48 pp.
- SIVORI, E., AND F. W. WENT. 1944. Photoperiodicity of *Baeria chrysostoma*. Bot. Gaz. 105: 321-329.
- TURESSON, G. 1922. The genotypical response of the plant species to the habitat. Hereditas 3: 211-350.
- . 1925. The plant species in relation to habitat and climate. Hereditas 6: 147-236.
- . 1930. The selective effect of climate upon the plant species. Hereditas 14: 99-152.
- WENT, F. W. 1943. Plant growth under controlled conditions I. The air-conditioned greenhouses at the California Institute of Technology. Amer. Jour. Bot. 30: 157-163.
- . 1944. Plant growth under controlled conditions. II. Thermoperiodicity in growth and fruiting of the tomato. Amer. Jour. Bot. 31: 135-150.

CULTIVATION OF EXCISED STEM TIPS OF ASPARAGUS IN VITRO¹

Shih-Wei Loo

NOTWITHSTANDING THE success of plant tissue culture in the past two decades, the cultivation of stem tips of plants has made little progress. As early as 1922, Robbins reported work on the culturing of stem tips of peas, corn, and cotton in a Pfeffer solution in the dark with and without the addition of 2 per cent glucose or levulose. The stem tips of peas and corn cultured for periods of 29 and 11 days, respectively, made considerable growth in the presence of carbohydrate and produced a number of roots. It was not until 1933 that another attempt was made by White to grow isolated stem tips, in this case of *Stellaria media*, in hanging drops. He showed that the stem tips developed well for a limited period in a modified Uspenski solution plus 2 per cent dextrose and 0.01 per cent yeast extract. The author concluded: "It has been possible to maintain such cultures actively growing for periods up to three weeks time. During this time there has occurred active cell division resulting in growth, accompanied by differentiation of the undifferentiated meristematic tissues into leaves, stems, and floral organs bearing apparently normal stomata." The results obtained by these two authors show that the stem tips either produced roots and became, therefore, ordinary seedlings or cuttings, or they ceased to grow within a very short period. In the fall of 1943, the writer found that the excised stem tips of asparagus can be cultivated *in vitro* through successive transfers in a synthetic medium. Such tips, unlike those used by Robbins and others, do not produce roots and continue to grow as excised stems. This paper presents a part of the experimental results.

MATERIALS AND METHODS.—The garden asparagus (*Asparagus officinalis* Linn.) was used exclusively in these experiments. The cultures were obtained as follows. Uniform seeds² were selected and germinated under sterile conditions in the dark at 26°C. for ten to twelve days. Stem tips 5 to 10 mm. in length were cut from the seedlings and transferred aseptically to individual culture tubes containing 10 cc. of nutrient. Each stem tip was supported by means of a small wad of pyrex glass wool so that only the lower end of the excised stem dipped into the nutrient solution. As a rule, fifteen stem tips were used for each treatment. All manipulations were carried out aseptically in an inoculation room sterilized by means of an ultraviolet lamp and sprayed with an antiseptic mixture.

The composition of mineral salts in the nutrient solution was essentially the same as that used by

¹ Received for publication September 6, 1944.

The author wishes to express his appreciation to Professor James Bonner for his many suggestions throughout this work and also to Professor F. W. Went for his keen interest and advice.

² The writer wishes to thank Mr. S. Winter of the California Packing Corporation for the seeds.

Bonner (1943) for isolated roots. This basic medium (BM) is prepared by dissolving in one liter of redistilled water 236 mgs. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 36 mgs. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 81 mgs. KNO_3 , 65 mgs. KCl , 20 mgs. KH_2PO_4 and 1.5 mgs. ferric tartrate. A number of physiologically active substances and various amounts of sucrose were added to the medium as described below.

Pyrex glass, chemicals of chemically pure grade, and redistilled water from a pyrex glass still were used exclusively. All of the cultures, unless otherwise stated, were kept in the air-conditioned culture room at 26°C., either in complete darkness, under diffuse daylight, or under continuous artificial illumination (40-watt daylight fluorescent Mazda lamp). The length increment of the stem tips was used as the criterion for growth.

POTENTIALLY UNLIMITED GROWTH.—On November 2, 1943, seven 5 mm. stem tips were transferred to petri dishes containing 20 cc. of nutrient solution (BM plus 2 per cent sucrose, 0.1 mg. each of thiamine and of pyridoxine per liter). They were placed in diffuse light near a north window. The stem tips changed their color from light yellow to green between the second and fifth day of cultivation, the change starting at the tip and progressing downward. Although the tips were not measured accurately, they made rapid growth (10 mm. or more) during a seven-day period. After seven days, tips 5 mm. long were removed from the initial stems and transferred to tubes containing 10 cc. of fresh medium. Thereafter, all the cultures were made in the following standard way: The stem tips were supported by a wad of glass wool in culture tubes containing 10 cc. of nutrient solution. Measurements of the lengths of the tips were made in each successive subculture after the second transfer. The results are summarized in table 1.

One of the seven stem tips was taken out at the end of the fourth transfer and compared histologically with the apical stem portion of a normal plant. The arrangement and cell size of meristematic tissue, as well as of the differentiated tissues, were substantially the same in the two cases. At the end of the fourteenth transfer another tip was sacrificed for the same purpose with similar results. It should be pointed out that some of the stem tips *in vitro* produced fewer cladophylls³ than normal plants, especially when the cultured shoots were subcultured at short intervals. This is probably an after-effect of the wound caused by cutting. Cultures kept in the light for long periods produced as many cladophylls as normal plants.

During the first nine transfers, the stems made an average of 0.5 to 2.0 mm. of growth in length per

³ The leaves of this plant are reduced to scale-like bracts and the leaf functions are taken over by special sterile twigs of limited growth known as cladophylls.

TABLE 1. *Growth of excised stem tips of asparagus in successive transfers in light (Exp. A-1).*

Transfer number	Length of transfer interval, days	Average growth in mm.	Average growth in mm. per day	Comments
1	7	BM + 2% sucrose; 0.1 per liter thiamine, pyridoxine, under diffuse light.
2	15	10.9	0.7	
3	5	10.0	2.0	
4	9	9.2	1.0	
5	7	8.7	1.2	
6	10	9.3	0.9	
7	11	4.6	0.4	
8	10	5.2	0.5	
9	22	11.0	0.5	
10	12	3.7	0.3	Same, but under continuous light and 3% sucrose.
11	16	1.4	0.1	
12	20	1.6	0.1	
13	23	1.9	0.1	Same, but under diffuse light and 2% sucrose.
14	11	1.1	0.1	
15	19	6.1	0.3	
16	15	3.2	0.2	
17	15	6.5	0.4	BM + 25 mgs. succinic acid, 100 mgs. (NH ₄) ₂ SO ₄ per liter.
18	9	3.8	0.4	
19	20	11.7	0.6	
20	20	6.2	0.3	Same, but 1% sucrose.

day. In the tenth transfer the sugar concentration was increased to 3 per cent, and the plants were at the same time placed under continuous artificial light. The growth rate dropped abruptly. In the fifteenth transfer the original conditions were restored, and the growth rate climbed slowly toward its original level.

Occasionally lateral buds developed on the cultured stems. When these lateral buds were excised and cultured, their growth resembled that of the main stem tips. In this way, the number of stem tips in the original culture series has been increased. It may also be possible to establish clones by this means. At present, however, no clone of more than three tips has been established. The original asparagus stem tips have been maintained through twenty successive transfers over a period of nine months and the cultures continue to grow at a rate comparable to the initial rate. It seems, therefore, safe to conclude that the growth of excised asparagus stem tips is potentially unlimited so far as the present cultures are concerned.

GROWTH IN LIGHT AND DARK.—In one series of

cultures, stem tips were grown under three different light conditions: diffuse daylight, continuous artificial illumination, and complete darkness. The results of this experiment are summarized in table 2. Stem tips in diffuse daylight produced more nodes than did those in continuous light, and the rate of elongation was more rapid. On the other hand, the number of cladophylls produced was the same under these two conditions.

The third lot of stem tips, that which was grown in the dark, made more growth than either of the others in the first transfer. These stems were subcultured through seven transfers, the data of which are given in table 3. The growth rate decreased from 3.6 mm. per day in the first transfer to zero in the seventh transfer. It is highly probable that substances needed for growth of asparagus stems and present in the original tip were depleted during the successive transfers. Stem tips growing in light, on the contrary, were able to synthesize at least small amounts of all of the substances necessary for unlimited growth, as shown in the preceding section. Stem tips grown in the dark were typically etiolated

TABLE 2. *Growth of excised asparagus stem tips in basic medium + 3% sucrose, 0.1 mg. per liter of thiamine and pyridoxine under different light conditions at 26°C. (Exp. A-18-1).*

Treatment	Average growth (15 tips) in 18 days (mm.)	Average growth in mm. per day	Average number of nodes per stem	Cladophylls
Normal day	36.5	2.3	7.7	numerous
Continuous artificial illumination..	24.7	1.4	4.4	numerous
Darkness	65.0	3.6	3.6	none

TABLE 3. Growth of excised asparagus stem tips in successive transfers in dark at 26°C., in basic medium + 4% sucrose and 0.1 mg. per liter of thiamine and pyridoxine. (Exp. A-18-2.)

Number of transfer	Length of transfer interval	Average growth in mm.	Growth in mm. per day
1 ^a	18	65.0	3.6
2	20	51.0	2.6
3	20	36.6	1.8
4	20	23.4	1.1
5	20	10.0	0.5
6	20	2.4	0.1
7	20	0.5	0.0

^a 3% sucrose was used in the first transfer.

in that they were elongated, produced relatively few nodes (table 2), and failed to produce cladophylls and lateral buds. Lateral buds did, however, develop occasionally after decapitation of the stem.

EFFECT OF SUGAR.—It has been shown above that sucrose is a suitable carbon source for excised asparagus stems. A series of experiments was carried out to determine the optimum concentrations of sucrose for growth in dark and in continuous artificial light. Typical results are shown in table 4 and figures 1 and 2. The stem tips grown in the dark were etiolated as were those described in the previous section. In the case of stem tips grown in the dark, growth increased with increasing sugar concentration during the first ten days of cultivation. The subsequent growth (second and third 10-day intervals) was, however, decreased or retarded by sugar concentrations higher than 4 per cent. It would seem, therefore, that 4 per cent is close to the optimum sucrose concentration for the growth of asparagus stem tips in the dark. Lower sugar concentrations were, however, optimal for the growth of stem tips in the light. Appreciable growth took place in the absence of added sugar. Concentrations of 0.5 and one per cent sucrose made the best growth; higher concentrations showed inhibition effects, especially when the concentration was raised above 3 per cent. Stem tips in nutrient media containing more than 2

per cent sucrose became deep violet in color owing to the formation of anthocyanins.

EFFECT OF ACCESSORY SUBSTANCES.—A number of physiologically active compounds were investigated for their effects on the growth of the stem tips *in vitro*. The compounds were added to basic medium containing 4 per cent sucrose. The composition of

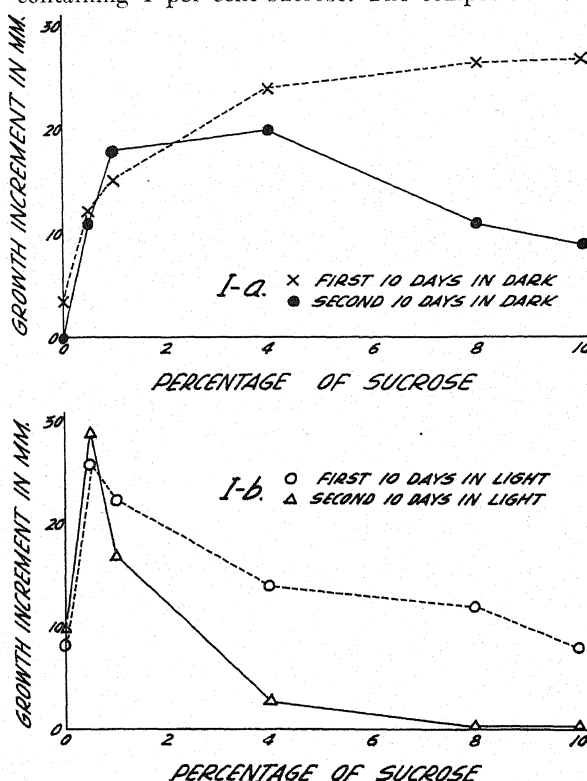


Fig. 1(a, b). The effect of sucrose concentration on the growth of excised asparagus stem tips (Exp. A-21).

the media and the results of these experiments are given in table 5. Among the various compounds, succinic acid, aspartic acid, and ammonium sulfate showed some effect in increasing the growth of the stem tips in the initial transfer. The effects of these three compounds were, however, not significant

TABLE 4. Average growth of asparagus stem tips in basic medium + thiamine and pyridoxine (0.1 mg. per liter) at 26°C. under light or in dark with different sucrose concentrations. (Exp. A-19).

		0%	1%	2%	3%	4%	6%	8%
Dark	10 days.....	0.5	8.6	9.1	9.7	20.5	23.4	28.3
	20 days.....	0.6	14.8	10.9	9.3	11.7	15.0	9.2
	30 days.....	0.8	18.1	13.4	10.0	11.8	5.3	2.2
	Total length	1.9	41.5	33.4	29.0	44.0	43.7	39.7
Light	10 days.....	5.7	26.0	20.0	18.5	15.0	13.0	14.3
	20 days.....	9.0	8.5	3.3	5.6	3.0	0.9	0
	30 days.....	8.8	5.5	4.5	4.4	1.2	1.1	0.4
	Total length	23.5	40.0	27.8	28.5	19.2	15.0	14.7

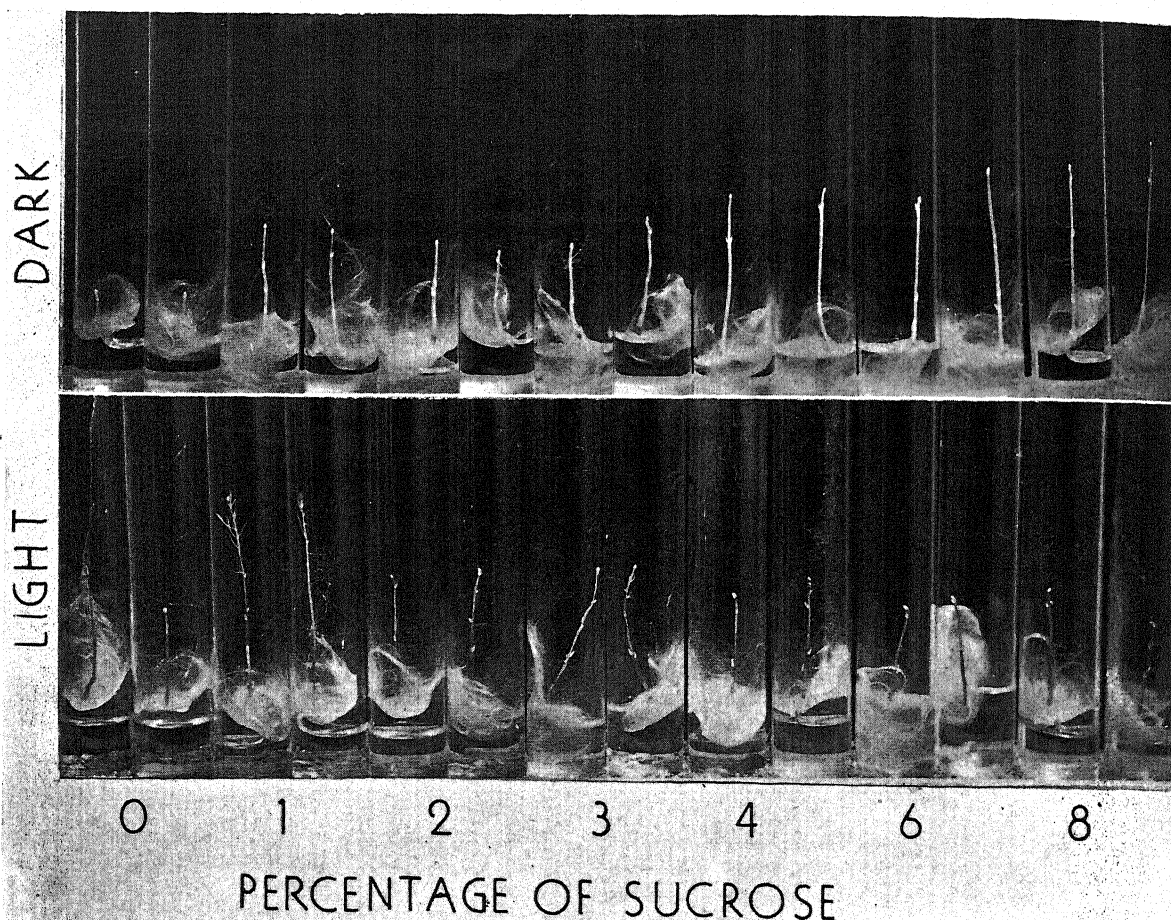


Fig. 2. Excised asparagus stem tips growing in the dark or in continuous light in media containing various amounts of sucrose (two samples shown per treatment). (Exp. A-19).

when the stem tips were maintained through successive subcultures.

Discussion.—It has been found possible to maintain and grow many plant tissues and organs in sterile culture. However, successful culture of the excised stem tip has not been reported previously. Attempts to grow excised stem tips usually have resulted in rooted cuttings or in complete cessation of growth within a short period. As shown in the present work, however, the excised stem tips of asparagus grow continuously as stems and produce roots very rarely if at all. This indicates that all of the materials needed for stem growth can be synthesized in the excised asparagus stem tip under suitable conditions. An exceptional case was found in which two of the stem tips (Exp. A-18-1, diffuse day light) produced adventitious roots, probably owing to hypocotyl tissue having been inadvertently included in the culture. The appearance of these roots increased the growth rate by three to four times over that found with rootless cultures. Normal intact seedlings under similar conditions grew 16 to 18 mm. per day. It is clear that, although excised asparagus stem tips can continue to grow through

numerous transfers over a prolonged period of time, this growth is at a slow rate compared to that of the normal seedling or compared to the rate attained by rooted asparagus stems. The effect of roots in increasing growth rate might be due to increased ability to the plants to take up water or nutrient from the medium or to the production by the root of one or more special stem growth-promoting substances (Went, 1938).

It is of interest to note that neither the compounds which have been shown to function as root growth substances (thiamine, pyridoxine, nicotinic acid, and glycine) nor the leaf growth substance, adenine, had any influence on the growth of excised asparagus stem tips.

From the results described above, it seems that there are two groups of factors or substances which promote the growth of excised asparagus stems. Substances of one group are produced in the stem in the presence of light but not in the dark; in the second group is the factor or substance transferred from the root system. The former substances are essential for the growth of the stem since, as shown above, the growth rate of stem tips in the dark decreased to

TABLE 5. Average growth of stem tips of asparagus in basic medium + 4% sucrose in dark at 26°C. (in mm.). 15 cultures in each treatment.

Culture number	Treatment	Exp. A-23		Exp. A-24 (Transfer interval 10 days)		
		10 days	20 days	First transfer	Second transfer	Third transfer
1	Control (BM + 4% sucrose).....	23.1	45.5	27.8	22.6	9.6
2	#1 + mixture (#3 to #10).....	39.0	61.0	32.2	21.0	11.2
3	#1 + glycine 3.0 mg. per liter.....	33.5	47.0	29.6	20.0	9.6
4	#1 + thiamine 0.1 mg. per liter.....	23.4	47.7	34.2	21.0	9.5
5	#1 + ascorbic acid 10 mg. per liter.....	34.6	53.0	33.7	16.0	6.1
6	#1 + nicotinic acid 0.5 mg. per liter.....	34.7 ^a	72.0 ^a	35.0	22.8	9.7
7	#1 + pyridoxine 0.1 mg. per liter.....	37.5	61.0	35.0	24.0	8.8
8	#1 + adenine 0.1 mg. per liter.....	34.7	53.7	41.7	22.5	9.6
9	#1 + succinic acid 25 mg. per liter.....	36.5	82.0	34.3	20.5	12.0
10	#1 + pantothenic acid 0.1 mg. per liter.....	38.2	66.0	37.0	21.2	10.7
11	#1 + biotin 0.001 mg. per liter.....	39.5	54.0	30.0	19.5	9.1
12	#1 + inositol 10.0 mg. per liter.....	27.4	47.0	24.0	20.0	11.1
13	#1 + aspartic acid ^b 100 mg. per liter.....	36.6	71.8	35.5	21.0	10.7
14	#1 + glutamic acid 100 mg. per liter.....	22.2	45.0	31.6	17.0	4.5
15	#1 + (NH ₄) ₂ SO ₄ 100 mg. per liter.....	42.8	71.5	29.0	27.6	16.4
16	#1 + (NH ₄) ₂ SO ₄ + succinic (#9 + #15).....	36.4	54.5	28.3	25.6	13.0
17	#1 + p-amino-benzoic acid 0.1 mg. per liter.....	29.8	52.0
18	#1 + asparagine 1 gm. per liter.....	29.6	65.0
19	#1 + indole-acetic acid 0.1 mg. per liter.....	21.4	51.5
20	#1 + indole-acetic acid 1.0 mg. per liter.....	9.4	20.0
21	#1 + indole-acetic acid 10.0 mg. per liter.....	5.2	9.5

^aOnly 3 out of 15 stem tips survived due to an accident.^bNeutralized.

zero during seven successive transfers. The root factor also greatly accelerates the growth of stems. This factor, if it is a chemical substance, may be synthesized in limited amounts by the stem, or it may be beneficial but not essential to stem growth.

SUMMARY

It has been shown that the excised stem tips of *Asparagus officinalis* were able to grow without the formation of roots in a synthetic medium *in vitro* under light. A series of cultures was maintained for nine months through twenty successive transfers. It is probable that this growth is potentially unlimited.

In darkness, growth of the excised stem tips was decreased through successive transfers and stopped entirely in the seventh subculture.

Relatively high sugar concentrations in the nutrient are required for growth in the dark. On the contrary, lower sugar concentrations favor growth in light.

A number of compounds have been tested of which succinic acid, aspartic acid and ammonium sulfate

promoted asparagus stem growth in the first transfer but not in the second or third transfers.

It appears probable that there are two groups of stem growth substances involved. One group is synthesized in the stem tip in the light and the other is produced in the root system.

WILLIAM G. KERCKHOFF LABORATORIES,
CALIFORNIA INSTITUTE OF TECHNOLOGY,
PASADENA, CALIFORNIA

LITERATURE CITED

- BONNER, JAMES. 1943. Further experiments on the nutrition of isolated tomato roots. *Bull. Torrey Bot. Club* 70: 184-189.
- ROBBINS, W. J. 1922. Cultivation of excised root tips and stem tips under sterile conditions. *Bot. Gaz.* 73: 376-390.
- WENT, F. W. 1938. Specific factors other than auxin affecting growth and root formation. *Plant Physiology* 13: 50-80.
- WHITE, P. R. 1933. Plant tissue culture; results of preliminary experiments on the culturing of isolated stem tips of *Stellaria media*. *Protoplasma* 19: 97-116.

VASCULARIZATION OF THE VEGETATIVE SHOOTS OF *HELIANTHUS* AND *SAMBUCUS*¹

Katherine Esau

ALONG WITH *Linum perenne*, considered in a previous article (Esau, 1943a), certain other plants were examined to determine the course of development of the first vascular elements in vegetative shoots. The present paper describes the differentiation of the first phloem and xylem in the shoots of *Helianthus annuus* L. and *Sambucus glauca* Nutt. These two plants were chosen because, having large leaves and trilacunar or multilacunar nodes, they strikingly contrast with *Linum perenne* which has small leaves and a single trace² per leaf.

MATERIAL AND METHODS.—The sunflower plants were raised in a greenhouse and the elderberry was growing wild in the vicinity of Davis, California. Five shoots of *Helianthus* and one of *Sambucus* were sectioned transversely, several others longitudinally. Ordinary paraffin and staining methods were used in preparing the slides. Some of the prepared slides were kindly supplied by Dr. R. H. Wetmore of Harvard University. In the examination of the sections the same procedure was followed as that outlined in connection with similar studies on flax (Esau, 1942, 1943a). In all illustrations the leaves were numbered beginning with the youngest at the apex of the shoot. If the leaves were paired at the nodes, the two members of the pair received different numbers, though the two leaves were of the same age. Such numbering was convenient for the marking of the traces within the stem. To simplify the descriptions of the shoots, all leaves including the youngest, are spoken of as "leaves" and the terms "embryonic leaves" or "leaf primordia" are rarely employed.

Helianthus.—Table 1 characterizes the five sunflower plants used for detailed study. All were young plants and showed no evidence of a change from the vegetative to the reproductive stage of development. Column 2 in table 1 gives the numbers of foliage leaves in the five plants. Besides these leaves each

¹ Received for publication, September 12, 1944.

² The term "trace" is applied throughout to each bundle which extends to a leaf.

plant had two cotyledons. As Priestley and Scott (1936) have reported, *Helianthus* does not have a stable type of leaf arrangement. The first few leaves are more or less decussate (fig. 2), later the phyllotaxis changes to some spiral system (fig. 9). Concomitant with this variability in leaf arrangement, the relation of the leaf traces to each other within the axial vascular skeleton shows no stable pattern. Each leaf usually has three traces, one larger, median and two smaller, lateral ones; occasionally there are more. (Leaf 15 of shoot 5 had four traces.)

The apex of a *Helianthus* shoot in vegetative stage does not form a cone; instead, it has a flat surface and the newly forming leaves arise as mounds on the margins of this surface (fig. 1). The internodes associated with the youngest leaves do not elongate for a number of plastochrones so that several leaves at the apex are attached at close levels. The axis expands laterally just below the apical meristem and, in longitudinal sections, appears to form broad shoulders on either side of it. The uppermost leaves rest upon these shoulders (fig. 1). In transverse sections the stem apex occurs, even at its highest level, not as a separate entity but is continuous with a mass of tissue formed by the united bases of several leaves (fig. 3).

The procambium was not studied in as much detail in the sunflower as in the flax (Esau, 1942) and the matter of the longitudinal course of procambial formation will not be commented upon in this paper. Procambial differentiation was usually evident in connection with leaves that were recognizable as small elevations above the apex; but no procambial strands were found that could have been interpreted as traces to future leaves whose primordia had not yet arisen. As in the flax (Esau, 1942), the procambium of the youngest organs is initiated through a series of longitudinal divisions. Figures 4 to 7 show the beginning of procambial differentiation in leaf 1 of the shoot depicted in figures 2 and 3; and figure 8 illustrates a portion of the procambial strand of

TABLE 1. Description of *Helianthus* shoots used in study of initial vascularization.

Plant number	Number of foliage leaves in plant	Number of leaves			Height in microns of youngest leaf with a mature sieve tube within	
		without mature vascular elements	without mature xylem elements	with mature phloem elements only ^a	trace in the axis	the leaf bundle above shoot apex
1	6	2	4	2	278	2800
2	8	2	4	2	0	960
3	8	1	4	3	120	760
4	10	4	6	2	0	1140
5	18	4	7	3	581	1134

^a The values in this column represent differences between the values in column 4 and those in column 3.

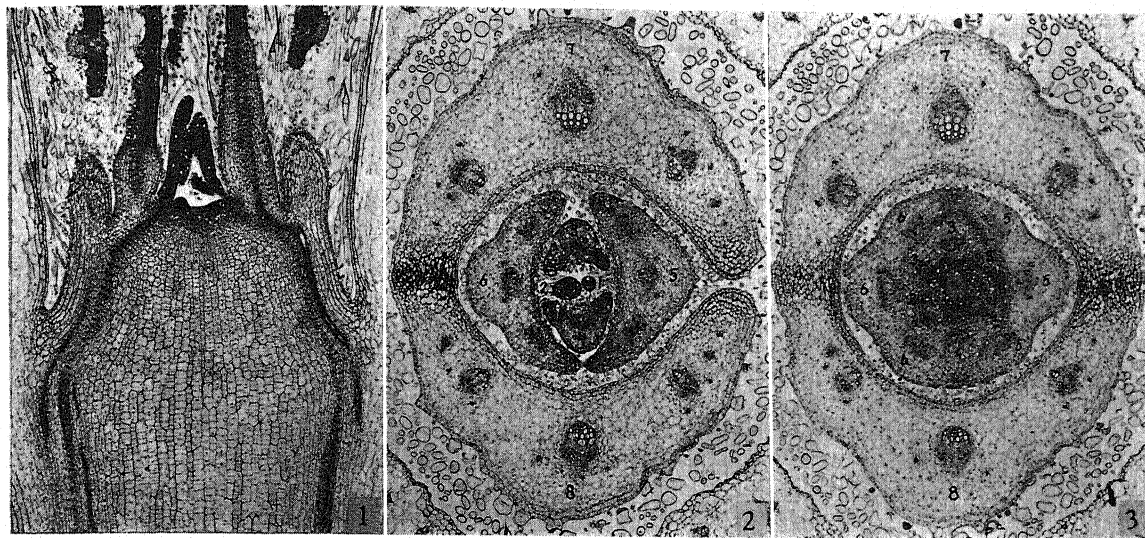


Fig. 1-3.—Fig. 1. Median longitudinal section of a *Helianthus* shoot showing the apical meristem and leaves in different stages of development.—Fig. 2 and 3. Transverse sections of a somewhat younger shoot than in figure 1. The section in figure 2 was taken 80 microns above the apex; that in figure 3, 8 microns below the apex. All figures $\times 35$.

leaf 2 of the same shoot. After these initial and some subsequent divisions the procambial cells remain small in their transverse dimensions while the adjacent parenchyma cells continue to enlarge in all directions. Thus gradually the procambial strands become clearly defined in transverse sections as islands of small cells among larger, non-procambial cells (fig. 15). While at first the longitudinal divisions occur in all planes, somewhat later the tangential longitudinal divisions begin to predominate so that when the first sieve tubes differentiate in a bundle the procambial cells show radial seriation (fig. 15). This feature becomes still more prominent when the future tracheary elements begin to enlarge and vacuolate: these elements occur in radial files separated from each other by xylem parenchyma (fig. 16). Thus as in many other vascular plants (see review by Esau, 1943b) the procambium in *Helianthus* resembles the cambium in forming derivatives in radial series.

The first sieve tubes are readily detected among procambial and adjacent phloem cells that are still rather densely cytoplasmic (fig. 15 and 16). With increasing vacuolation of the vascular bundles the sieve tubes become less distinct, but one can in a doubtful instance follow a given element through a series of sections until a sieve plate is encountered. Sometimes the oil ducts resemble sieve tubes but these are located outside the vascular bundles (fig. 16). The definitions of mature and immature sieve-tube and xylem elements have been given previously (Esau, 1942). The conducting elements of the xylem are referred to throughout as "tracheary" or "xylem elements," though they appear to be vessel elements and have been so identified by Priestley and Scott (1936).

The direction of differentiation of the first vascu-

lar elements in *Helianthus* is generally similar to that established for flax (Esau, 1943a). Xylem elements were found to mature first in the leaf and the upper parts of the traces, then in the lower parts of the traces that were connected with some other, older traces. In all five plants the first xylem elements formed long vertical series so that the exact place of the initial xylem maturation could not be determined as closely as in the flax. (Compare fig. 14 of this paper with fig. 9 in Esau, 1943a.) Some of the lateral traces had shorter series of elements and these extended through the base of the leaf and the uppermost part of the trace. These observations suggest that, once initiated, xylem maturation progresses very rapidly through a considerable length of the leaf bundle and trace and that this differentiation is acropetal within the leaf proper, and basipetal within the axis.

The first phloem was seen to differentiate acropetally from some trace of an older leaf into that of a younger, and through the young trace into the leaf. As in flax, however, the maturation of the first phloem elements was not entirely continuous: in some traces the longitudinal series of sieve-tube elements consisted partly of immature elements interpolated among the mature ones (fig. 14). Table 1 (columns 6 and 7) shows that the youngest leaves having the first mature phloem elements in the trace or in the leaf proper vary greatly in size in different plants. (The height of each of these leaves was measured between the apex of the leaf and its base, where it was attached to the stem.) Judging by columns 3 to 5, there is also some variation in the distance between the apex and the leaves which show the first mature vascular elements. (In columns 3 and 5, a leaf that had a mature sieve tube in its trace but not yet in the leaf proper was counted as one

having mature phloem elements; e.g., leaf 5 in fig. 9-13.) In other words, the number of plastochrones

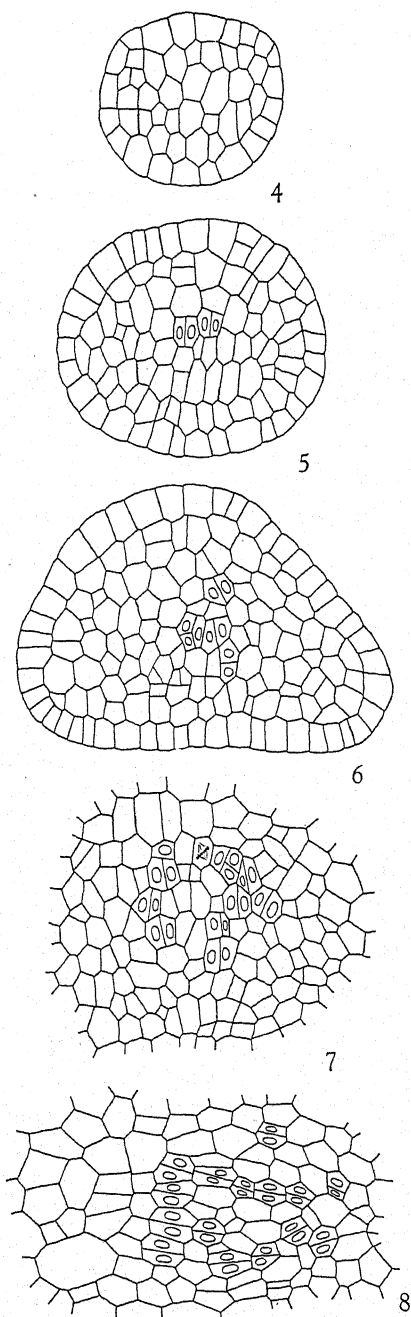


Fig. 4-8.—Fig. 4-7. Successive transverse sections through the youngest leaf (leaf 1) of the *Helianthus* shoot illustrated in figures 2 and 3. The four sections were taken the following number of microns below the apex of the leaf: 21, 42, 63, and 84. The lowermost of these sections occurred beneath the attachment of leaf 1 to the axis.—Fig. 8. Transverse section through the same shoot as above but showing the procambial strand of leaf 2 in the region where this strand was diverging into the leaf. In the figures 5 to 8 the nuclei indicate the cells that resulted from recent longitudinal divisions. All figures $\times 350$.

during which a leaf remains free of one or the other or of both kinds of vascular elements varies in different plants. In the group of sunflower plants used in this study, leaves developed no vascular elements in their procambial bundles during 1 to 4 plastochrones; they had mature phloem elements not accompanied by xylem during 2 to 3 plastochrones; and they were lacking xylem during the total of 4 to 7 plastochrones (column 3 plus column 5). One feature is consistent: within a given leaf the first sieve-tube elements mature before the first xylem elements. The common pattern is as follows: the vascular elements mature first in the median trace, then in the lateral ones; and the first xylem matures in the median trace after some phloem appears in the lateral traces (fig. 14).

Figures 2 to 14 give details concerning some of the plants described in table 1. The photomicrographs in figures 2 and 3 were taken from plant 3 and give an idea of the relative degree of differentiation of the leaves on this plant. (The leaf portions occurring in the corners of these photographs belong to the cotyledons.) Leaf 1 showed procambial differentiation but no mature vascular elements (fig. 4-7). The median procambial strand of leaf 2 had, in its lower axial portion, a mature sieve tube that was continuous with the phloem of a lateral trace of leaf 6. The lateral strands of leaf 2 were not yet differentiated, though some longitudinal divisions were discernible in the future positions of these strands. The median strands of leaves 3 and 4 had mature sieve tubes in the axis and in the leaves proper, the lateral strands in their axial portions only. The median strand of leaf 3 with its first sieve tube is depicted in figure 15. Leaf 5 had five mature sieve tubes and the first mature xylem elements (fig. 16) that were not yet connected with the xylem in the axis. The xylem of leaf 6 was also discontinuous.

Figures 9 to 13 illustrate the trace relationships at successive levels of the axis and the course of the first xylem and phloem in plant 5. Though in the younger leaves the numbers of dots represent the actual numbers of sieve tubes visible in the sections, in the larger traces the counts were only approximate. This is particularly true of the lower levels of the axis where obliteration of the first phloem elements had occurred in the larger traces. Such areas of obliteration have been left blank in the large bundles of figures 12 and 13. After the obliteration of the sieve tubes sclerification of cells occurs in the primary phloem region and the sclerenchymatous bundle caps characteristic of *Helianthus* are formed. Thus, as in many other plants, the bundle caps in *Helianthus* originate as parts of the primary phloem and not as parts of the so-called "pericycle."

In plant 5 the leaves 1 to 10 were the following numbers of microns high: 10, 112, 238, 350, 581, 1134, 1211, 1617, 2380, and 3024. The apices of the other eight leaves were not available on the slides. The procambial strand of leaf 1 was not identified with certainty. Leaves 2 to 4 had three

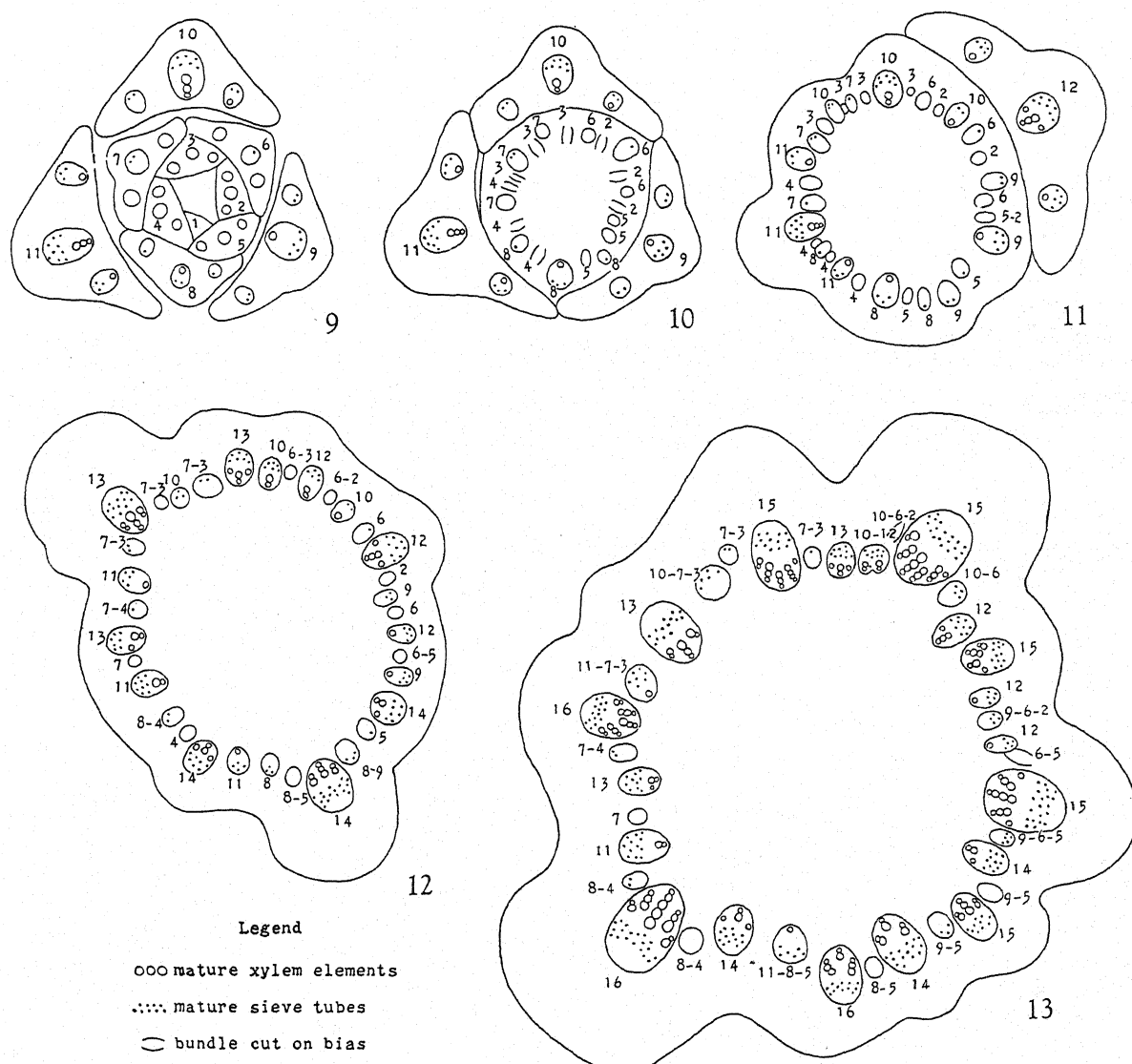


Fig. 9-13.—Successive transverse sections through a *Helianthus* shoot showing the arrangement of the vascular bundles.—Fig. 9. Section through the apex and 11 leaves surrounding the apex.—Fig. 10-13. Sections through the axis (and the attached leaf bases) taken, respectively, the following number of microns below the apex: 56, 203, 574, and 1635. All figures $\times 42.5$.

procambial strands each and no mature vascular elements. The procambial strands were connected with some older traces in the axis. At the level of figure 9 the procambial strands of leaf 5 were free of mature vascular elements; farther down a sieve tube occurred in the median trace 5 (fig. 11). Thus in plant 5, leaf 5 was the youngest with a mature phloem element in the trace (table 1, columns 3 and 6). Leaf 6 was the youngest having a mature sieve tube within the leaf proper (table 1, column 7); and leaf 8, which was 1617 microns high, the youngest with some mature xylem (fig. 9).

The numbers of the mature vascular elements in the various leaf bundles were about the same in the section cut at the apical level (fig. 9) and in those

obtained within the first 200 microns below the apex (fig. 10). Below the node of leaf 11 (fig. 11) mature sieve tubes appeared in some traces that lacked these elements at higher levels. Thus mature phloem elements occurred here in the median trace of leaf 5 and in the two laterals of leaf 7. The cathodic³ lateral of leaf 2 was merged, at this level, with the anodic³ lateral of leaf 5. The cathodic lateral of leaf 3 appeared here in the form of two branches flanking the median trace of leaf 10. Similarly, the anodic lateral of leaf 4 branched above the gap of the anodic lateral of leaf 11.

³ "Anodic" turned toward, "cathodic" turned away from the course of the genetic spiral along which the leaves are arranged.

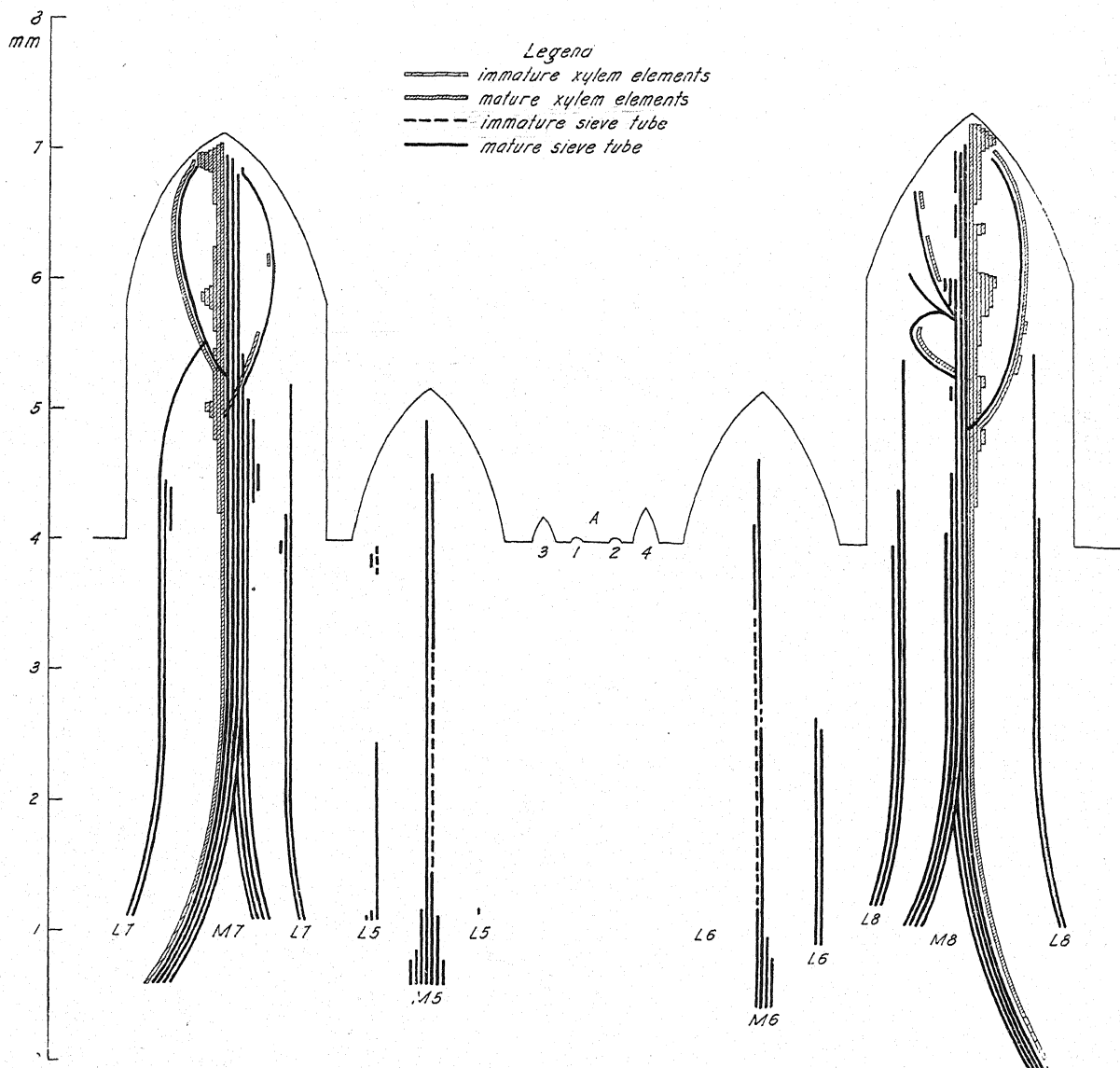


Fig. 14.—Diagram illustrating the longitudinal course of development of the first phloem and xylem in a *Helianthus* shoot. Further explanations in text.

Between the levels of figures 11 and 12 the following changes in bundle orientation were perceived. The traces of the leaves 12 to 14 became parts of the vascular cylinder. The anodic laterals of leaves 6 and 2 and a branch of the cathodic lateral of leaf 3 fused into one bundle; farther down this compound (sympodial) bundle branched above the anodic lateral of leaf 12 (fig. 12, bundles 6-3 and 6-2 on the sides of bundle 12). The cathodic lateral of leaf 6 branched above the cathodic lateral of leaf 12 and one of these branches fused with the sympodium that included the anodic lateral of leaf 5 and the cathodic lateral of leaf 2. One branch of the anodic lateral of leaf 4 fused with the median trace of leaf 8; the median of leaf 4 with the cathodic lateral of leaf 8; and the cathodic lateral of leaf 4 with a branch of the anodic lateral of leaf 7. The two

branches of the anodic lateral of leaf 7 appeared above the gap of the anodic lateral of leaf 13. The median trace of leaf 7 and the anodic lateral of leaf 3 fused and farther below the compound bundle branched above the median trace of leaf 13. The median trace of leaf 3 and one branch of the cathodic lateral of the same leaf merged with the cathodic lateral of leaf 7; as was mentioned previously, the other branch of the cathodic lateral of leaf 3 fused with the anodic lateral of leaf 6. The anodic lateral of leaf 8 branched above the median trace of leaf 14 and the branches fused, one with the cathodic lateral of leaf 9, the other with the cathodic lateral of leaf 5. The median trace of leaf 8 shows no xylem in figure 12; the first and only series of mature xylem elements of leaf 8 ended about 500 microns below the stem apex. The median trace of leaf 2 is still dis-

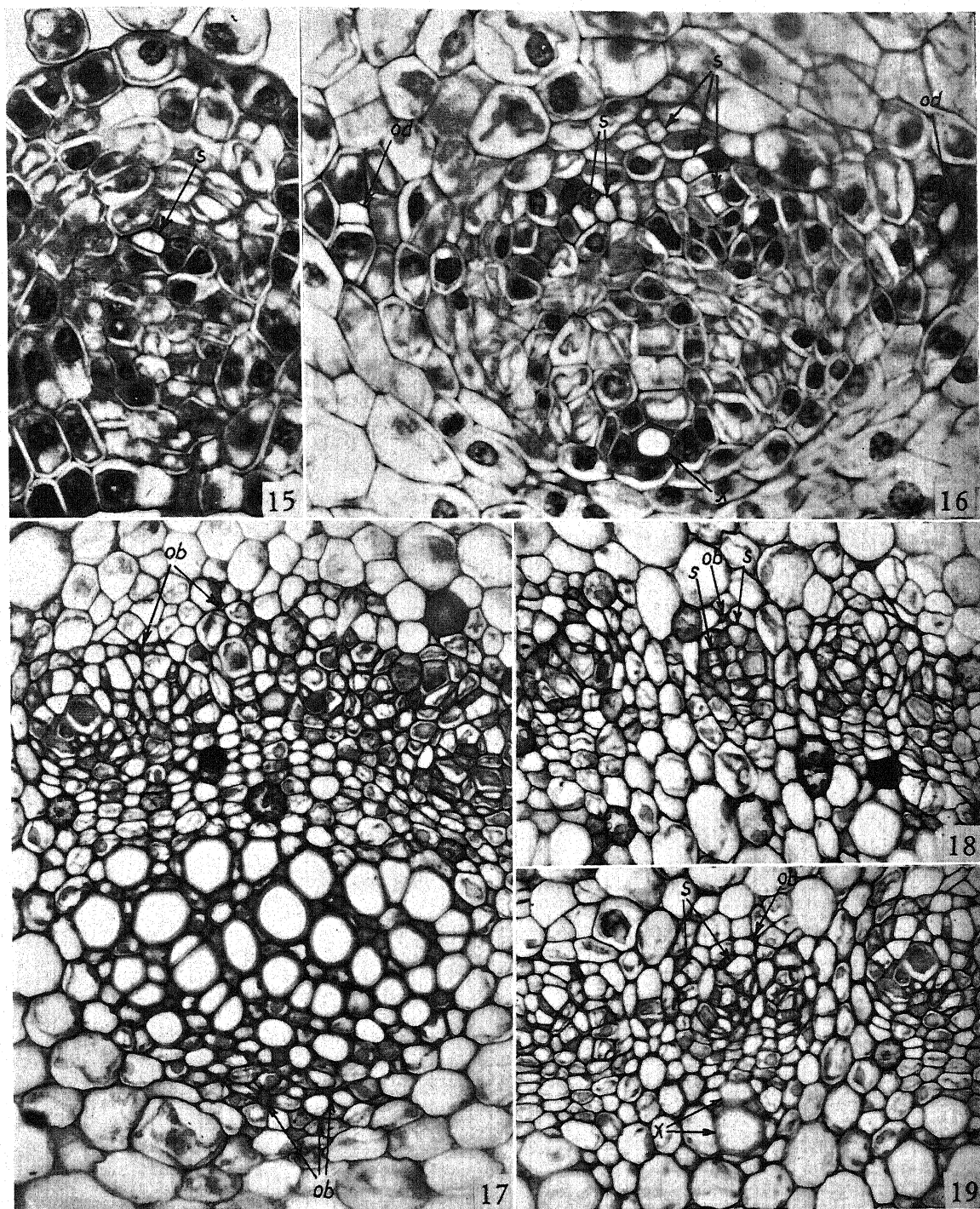


Fig. 15-19.—Fig. 15 and 16. Transverse sections of the median vascular bundles of leaves 3 (fig. 15) and 5 (fig. 16) of the *Helianthus* shoot illustrated in figures 2 and 3. $\times 760$.—Fig. 17-19. Transverse sections of vascular bundles taken from one section of a young *Sambucus* stem. $\times 360$. Details are: ob, obliterated xylem and phloem elements; od, oil duct; s, sieve tube; x, xylem element.

crete at the level shown in figure 12, while those of leaves 3 and 4 are fused with some older traces.

The details presented above will suffice to illus-

trate the complexity of the vascular system of a plant having more than one trace per leaf and, besides this, undergoing a change in phyllotaxis during

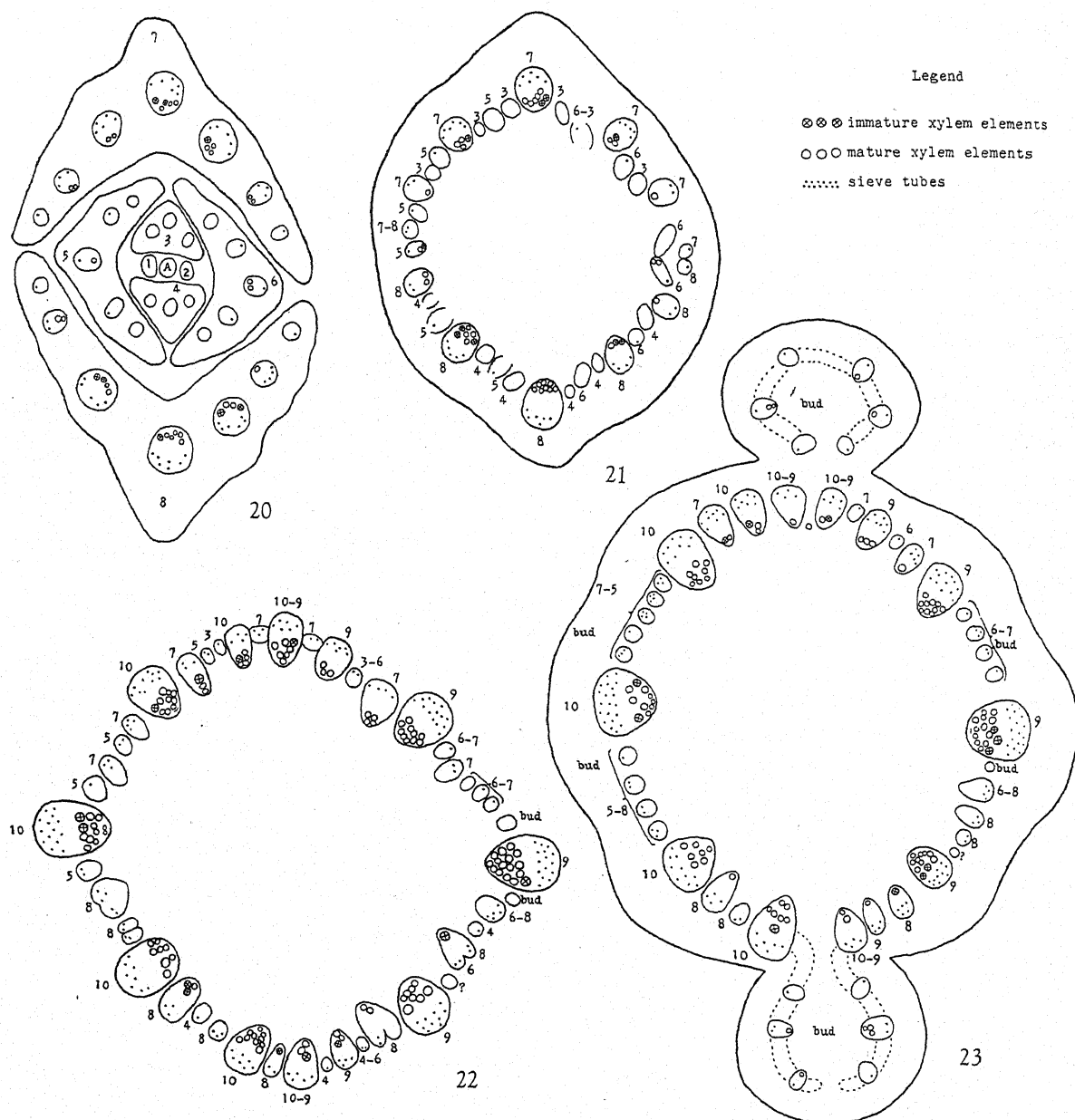


Fig. 20-23.—Successive transverse sections through a *Sambucus* shoot showing the arrangement of the vascular bundles.—Fig. 20. Section through the apex (A) and 4 pairs of leaves surrounding the apex.—Fig. 21-23. Sections through the axis taken, respectively, the following number of microns below the apex: 290, 1100, and 1930. All figures $\times 37$.

its development from seedling to maturity. Attention to such details is indispensable in a study of the course of the first vascular elements.

Since the earliest xylem and phloem elements are the primary objects of the present study, only the youngest traces will be considered in the stem regions below that shown in figure 12. The median procambial strand of leaf 2 fused with the sympodium consisting of the anodic lateral of leaf 9 and a part of the cathodic lateral of leaf 6. From this sympodium (9-6-2 in fig. 13) the first sieve tube would have differentiated into the median trace of leaf 2. The median of leaf 3 would have received its

sieve tube from the 7-3 (fig. 12) or the 10-7-3 complexes (fig. 18). The purely procambial traces of leaf 4 were fused with the traces of leaves 7 and 8 at the level depicted in figure 13. The laterals of leaf 5 that also lacked mature vascular elements are parts of compound bundles with the traces from leaves 2, 6, 8, and 9 in figure 13. The median trace of leaf 5 had a mature sieve tube for a length of about 800 microns which shows in figure 12. At levels where the median trace of leaf 5 was partly merged with the cathodic lateral of leaf 9 (the sieve tubes of the two traces were not fused at these levels) the sieve tube of leaf 5 consisted of immature elements.

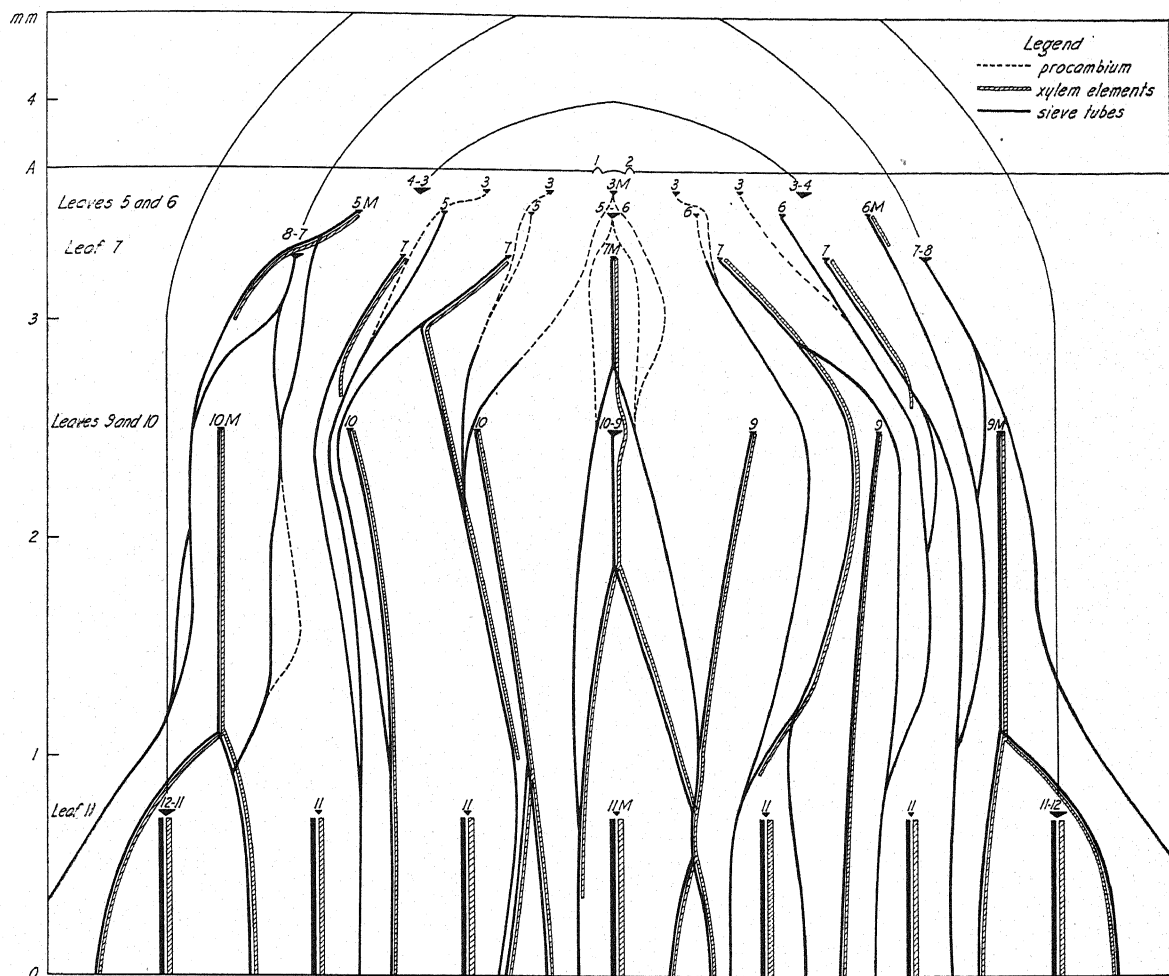


Fig. 24.—Diagram illustrating the longitudinal course of development of the first phloem and xylem in the same *Sambucus* shoot as illustrated in figures 20–23. Further explanations in text.

The same sieve tube could be followed in the compound bundle 9–5 and its anodic branch appearing on one side (upper side in fig. 13) of the cathodic lateral of leaf 15. It was immature through 50 microns, mature farther below through 648 microns, then again immature down to the level where the 9–5 branch containing this sieve tube merged with the anodic lateral of leaf 14 (3910 microns below the apex). The sieve tube of the median trace of leaf 6 was mature throughout, and at the level of figure 13 was fused with a sieve tube of the cathodic lateral of leaf 10. The sieve tubes of the traces of leaves 7 and 8 had mature connections with the phloem of older traces. The sieve tube of the compound bundle 8–5 (actually the sieve tube of the anodic lateral of leaf 8) was immature in its lower levels (bundle 8–5 in fig. 12 and 13). The xylem elements of the 9–6–5 complex (xylem of the median trace of leaf 9) were immature at the 1635 micron level (fig. 13) and had no secondary walls farther below. The mature xylem of the cathodic lateral of leaf 10 continued part way down below the level

shown in figure 12; that of both laterals of leaf 11 and of the complex 10–12 (median of leaf 10 and anodic lateral of leaf 12) reached somewhat below the level of figure 13. Thus three leaves, 8, 9, and 10, which were, respectively, 1617, 2380, and 3024 microns high, had no mature xylem connections with the older leaves within the 1.5 cm. of stem sectioned from plant 5.

Using the data from the serial transsections of plant 4 the diagram in figure 14 was prepared. To simplify the picture the leaves were placed alternately on the two sides of the apex and the features of decussation and spiralization in the leaf arrangement were disregarded. Since the leaves represented in figure 14 were all joined at the level of the apex, in the diagram they are shown connected by a horizontal line passing through the apex. Longitudinally, the parts of the diagram were drawn to scale; horizontally, only the convenience of arrangement of parts on the diagram was considered. The interconnections of traces are not shown because the vascular strands of leaves 5 to 8 were connected with the

traces of leaves older than the eighth—leaves not represented in the figure. The vascular bundles are shown interrupted in the basal part of the diagram at levels where they were connected with the traces of the older leaves. For example, the median traces of leaves 5 and 6 (M5 and M6) were connected with some other traces below the 1 mm. mark on the diagram. The laterals of these leaves (L5 and L6) were joined to other traces at somewhat higher levels than the medians. The leaves 1 to 4 had no mature vascular elements and their procambial bundles were not represented in the diagram.

In leaves 5 and 6 some sieve tubes in the median bundles had differentiated in the region from the union of these bundles with that of other traces to nearly the apices of these leaves. In M5 only one sieve tube was mature through the whole length, the other consisted partly of immature elements. In M6 both sieve tubes were partly immature. At their bases the traces had more mature sieve tubes than higher up. The first sieve tubes of the laterals (L5 and L6) began to differentiate later than those of the median traces as evidenced by their shorter lengths in the diagram; and one lateral of each leaf was ahead of the other in phloem differentiation. Of particular interest to the writer were the few immature sieve-tube elements in the left lateral of leaf 5 located below the base of the leaf and not connected—even by immature elements—with the sieve tubes of the same traces located lower in the axis. The presence of these elements indicates that, at least occasionally, sieve tubes may be initiated in isolated portions of a trace like the tracheary elements.

The leaves 7 and 8 had xylem in the median bundles (M7 and M8) but this xylem was connected with that of older traces by immature elements. In this diagram the xylem and phloem were recorded in detail throughout, including the leaf blades. The diagram shows that considerable xylem was present in the blades of leaves 7 and 8, and that none of this xylem had a mature connection with the xylem of the axis. The phloem of leaves 7 and 8 was considerably more mature than that of leaves 5 and 6. The slower differentiation of the laterals as compared with the median traces is illustrated by leaves 7 and 8 also.

Sambucus.—The development of the vascular system of the elderberry shoot is illustrated in figures 20 to 24. The vascular bundles of this shoot were followed from the apex down through five entire internodes and somewhat below the sixth node. Figure 20 illustrates the decussate arrangement of the leaves (no leaflets occurred at this level, only the rachae of the compound leaves) characteristic of the genus. This section was cut at the level *A* in the diagram (fig. 24), that is, through the terminal portion of the shoot, and the shoot apex (*A*) appears in the center of figure 20. The largest leaves in this shoot had, at their bases, seven bundles each. Within the axis the median and the four nearest lateral bundles were prolonged in the axis as five separate

traces each diverging from a separate gap. The two members of a pair of leaves were fused at the base and here the most peripheral lateral bundles of the two leaves were united into a compound bundle. Each compound bundle was continued as a single strand within the axis. This feature is illustrated to the right and left in figure 21 (bundles 7 and 8) and above and below in figure 22 (bundles 9 and 10). Figure 21 was cut below the node of leaves 7 and 8, figure 22 below that of leaves 9 and 10, and figure 23 above the node of leaves 11 and 12 and includes sections of the axes of the two buds that occurred in the axils of leaves 11 and 12.

The diagram in figure 24 represents the vascular system of one-half of the same shoot as in figures 20 to 23. It is drawn as though the stem were cut in halves through the median traces of leaves 5, 6, 9, and 10, and the half bearing leaves 3, 7, and 11 was flattened out. Thus all of the traces of leaves 3, 7, and 11 are represented in the diagram, and only half of those of leaves 5, 6, 9, and 10. The traces of leaves 4, 8, and 12 are represented only by the members of the compound bundles 3-4, 4-3, 7-8, 8-7, 11-12, and 12-11. The arcs above are diagrammatic outlines of leaves 3, 7, and 11. The median traces of these leaves (identified by the letter M) appear in the center of the figure, those of leaves 5, 6, 9, and 10 at its sides. The triangles at the upper ends of the traces indicate the approximate levels at which these traces diverged into the leaves. Among these triangles the larger ones mark the compound bundles consisting of the peripheral traces of pairs of opposite leaves (bundles 5-6, 7-8, 8-7, 10-9, 11-12, and 12-11). As in the *Helianthus* diagram, the bundles were drawn to scale longitudinally; and the transverse distances between the traces were made such that the bundles could be conveniently accommodated in the diagram. The traces of the axillary buds were omitted in the diagram; nor are the procambial strands of leaves 1 and 2 represented in this figure. No attempt was made to draw the actual number of the sieve tubes and of the series of tracheary elements, as was done in the *Helianthus* diagram, and only the axial parts of the bundles are shown.

The two youngest leaves 1 and 2 were each 30 microns high and showed longitudinal divisions in their median portions (as seen in transverse sections). Through these divisions the median procambial strands were being organized. Leaves 3 and 4 had attained the height of 460 microns. Each had three procambial strands at the level of the apical meristem (fig. 20), and five traces farther below. The compound bundles 4-3 and 3-4 (fig. 24) were not discerned with certainty. In figure 21, above, the median trace of leaf 3 appears as two bundles on the sides of the median trace 7; to the right and left of the median, four more traces of leaf 3 appear among other bundles (one lateral of leaf 3 is fused with a trace to leaf 6). The traces of leaf 4 show similar distribution on the opposite side of the stem. About midway between the nodes 7-8 and 9-10, one

of the branches of each median trace of leaves 3 and 4 had mature sieve tubes (fig. 22 and 24); these continued downward to their connection with the sieve tubes of the older traces. Each of the third pair of leaves (leaves 5 and 6) had sieve tubes in the median and two lateral traces and xylem elements in the median. The sieve tubes were connected with the sieve tubes of the older traces; the xylem elements reached only to slightly below the insertion of the leaves (5M and 6M in fig. 24). Leaves 7 and 8 had sieve tubes in all traces and these were connected with the phloem strands from the older leaves. The youngest laterals in leaves 7 and 8 were still lacking mature xylem elements, and only the xylem of the median strands of these leaves had mature connections with the older traces. Some of the xylem of leaves 9 and 10 was also discontinuous below the levels depicted in figure 24. The traces of leaves 11 and 12 were available in their upper levels only.

Figure 24 illustrates the continuous acropetal differentiation of the phloem elements and the discontinuous initiation of the xylem and its basipetal course in the axial prolongations of the leaf bundles. It also demonstrates that the first xylem and phloem of a given trace may be connected with older traces at different levels. Thus the xylem of 7M is connected with trace 10-9 at a higher level than the phloem of 7M. Furthermore, the first xylem of 7M was not branched above the gap of the strand 10-9, whereas the phloem strand was forked here (fig. 24). The later-formed xylem (but still protoxylem) branches at similar levels as the collateral phloem. Thus in figure 23, above, the trace 10-9 appears as two branches (above the gap of leaf 11 and its bud) including the phloem and most of the xylem. One xylem element, however, appears between these two bundles: this element belonged to a series that continued straight down to the next lower trace of leaf 11 (this xylem is not represented in figure 24). In other words, the first xylem elements differentiated through the so-called "gap." Since such elements are parts of the protoxylem they are soon obliterated and are not discernible in older stem regions.

As figures 20 to 24 indicate, the trace interconnections do not occur according to a very stable pattern. For example, the gap over 11M (and its bud) occurs between the branches of the compound bundle 10-9 (fig. 23, above, and fig. 24). The opposite 10-9 bundle, however, is not branched above the gap of leaf 12 and its bud (fig. 23, below). A given trace may be branched at one level and appear as one bundle above and below the branching (fig. 24, phloem bundle on the extreme left). Because of such complexities the vascular tissues may become regrouped into bundles that are not identifiable as certain individual or compound traces. Thus the groups of bundles marked by brackets in figures 22 and 23 are branches of compound bundles formed by the traces of the leaves whose numbers appear outside the brackets and by the traces of the axillary buds of leaves 9 and 10. Nevertheless every bundle in this

series was related directly or indirectly to some existing leaf or bud.

Figures 21 to 23 show that the bundles within the young axis vary greatly in size. These variations, of course, depend on the differences in age of the leaves to which these bundles are related and also on the differences in size and, consequently, in time of initiation, between traces of the same leaf. Figures 17 to 19 illustrate one large and two small (the central bundles in photographs 18 and 19) bundles from the same cross section of an internode that was comparable to the seventh internode of the shoot represented in figures 20 to 23. The largest bundle contains considerable xylem and phloem; the bundle in figure 19 shows two xylem elements and some phloem, and the one in figure 18 contains phloem only. The bundle in figure 17—a large lateral trace of a leaf attached at the nearest node above—was initiated early in the stem portion where it occurred and therefore it contains a high proportion of proto-phloem and protoxylem. In both tissues many conducting elements have been obliterated. The small bundles in figures 18 and 19, which began to differentiate later than the bundle of figure 17, have some protophloem with obliterated sieve tubes. The bundle in figure 19 shows a small amount of xylem, whereas that in figure 18 has none. The vascular meristem present in the bundles in figures 17 to 19 is still in procambial state. The procambium of *Sambucus* like that of *Helianthus*, shows an early predominance of tangential longitudinal divisions, especially in the xylem region. In the elderberry, however, the successive tracheary elements do not follow each other in the same radial files of cells as in the sunflower, and therefore the origin from a radially seriated meristem is obscured in the former (fig. 17).

The *Sambucus* series gives some information on the course of development of the bud traces. The buds in the axils of leaves 9 and 10 had procambial strands only. The bundles of each bud were prolonged in the underlying internode of the main axis as two traces that, at certain levels, fused with the trace complexes related to leaves 5, 6, 7, and 8 (fig. 22-23). The traces of the buds in the axils of leaves 11 and 12 had some xylem and phloem. The xylem was not yet connected with any in the main axis, but ended blindly shortly below the node of leaves 11 and 12. The sieve tubes were continuous to the end of the series of sections, 3 mm. below the attachment of the buds to the main axis. In figure 23 the buds show groups of bundles. The three bundles of each group are traces to one leaf—the leaf of the first pair of leaves on the lateral shoot. Each group of three traces is combined below into one bundle, the branch trace.

DISCUSSION.—Since the present writer (Esau, 1943b) reviewed the literature on the differentiation of the primary vascular tissues, several articles on this subject have appeared in print (Crafts, 1943a, 1943b; Engard, 1944; Esau, 1943a; Reeve,

1942). These articles and the present study agree that the first phloem elements differentiate acropetally from the axis into the leaf; but not all of the articles cited describe the entire course of the first phloem elements, that is, the course between the highest position in the shoot and the place of connection with the phloem of other traces. By examining the first sieve tubes throughout their entire length the present writer has found in both the flax (Esau, 1943a) and the sunflower that the first phloem elements tend to differentiate continuously and acropetally from the axis into the leaves, but that this "continuous differentiation" is sometimes followed by a "discontinuous maturation"; that is, in some traces, series of immature sieve-tube elements are interpolated between series of mature ones. Furthermore, one of the shoots of *Helianthus* even indicated that the sieve tubes may be initiated (that is, the mother cells of their elements may become recognizable as such) in isolated parts of a given trace (fig. 14, lateral trace of leaf 5).

As a rule, in a growing shoot the first protophloem elements differentiate before the first protoxylem elements in a given bundle (Crafts, 1943a, 1943b; Engard, 1944; Esau 1938, 1943a, 1943b, 1943c; Reeve, 1942; Sharman, 1942). Bond's (1942) contrasting view that in the tea plant the protoxylem elements precede the appearance of the "first recognizable sieve tubes of the protophloem" needs further evidence to prove that the "first recognizable sieve tubes" are actually the "first to differentiate" in a given bundle.

The differentiation of the first phloem elements in advance of the first xylem elements in a given bundle means that at the shoot apex the first sieve tubes (or sieve cells) differentiate one or more plastochrones before the first tracheary elements. Thus, beginning at the apex, there are one or more leaves without mature vascular elements; one or more with sieve tubes only; then follow leaves with both xylem and phloem (table 1). In the flax (Esau, 1943a) the number of leaves of each type varied in different shoots in relation to the phyllotaxes of these shoots. In agreement with the concept of Priestley *et al.* (1937), the flax leaves could be roughly divided into series numerically related to the denominator of the phyllotaxis fraction. In their studies of *Helianthus*, Priestley and Scott (1936), using a $3/8$ -phyllotaxis type of a shoot, interpreted as the first cycle of leaves the eight primordia aggregated at the apex and lacking axillary buds. The eight primordia of the next cycle were successively associated with internodes of increasing length. The differentiation of the first phloem was well within the first cycle of leaves, while the first xylem tended to appear toward the end of this cycle. The oldest sunflower plant of the present study (plant 5, table 1, and fig. 9-13) fits the above description though it did not quite reach the $3/8$ -phyllotaxis stage of development. The other plants (plants 1-4, table 1) were younger than plant 5 and showed shorter cycles as measured by

the number of leaves without mature vascular elements. According to the concept of Priestley *et al.* (1936, 1937), shorter cycles would be expected in young *Helianthus* plants because the earlier leaves of *Helianthus* are more or less decussate and only the later ones assume a spiral arrangement and become more crowded at the apex. The *Sambucus* shoot with its decussate leaves showed the following sequence: no vascular elements during one plastochrone (2 leaves); sieve tubes differentiating acropetally along the traces into the leaves during the second plastochrone (two leaves); xylem elements appearing in the basal part of the leaf during the third plastochrone (two leaves). A summing up of the present writer's data on *Nicotiana* (Esau, 1938), *Linum* (Esau, 1943a), *Zea* (Esau, 1943c), *Helianthus* and *Sambucus* suggests that the greater the crowding of leaves at the apex, the slower is the vascular differentiation in the leaves. Seasonal and other environmental conditions could be expected to affect the rates of differentiation within a given type of shoot.

The latest studies on the vascularization of shoots (Crafts, 1943a, 1943b; Engard, 1944; Esau, 1943a; Reeve, 1942) continue to agree with the older literature (see Esau, 1943b) that the first tracheary elements are initiated in one or more isolated positions within the leaf or in the upper part of its trace, and subsequently differentiate downward into the axis and upward into the leaf. During the acropetal differentiation of the xylem and phloem within the leaf proper the xylem tends to approach the apex before the phloem. These observations were substantiated by the data obtained from *Helianthus* and *Sambucus*.

Though procambial differentiation was not considered in detail in the present study, the matter of initiation of the procambium merits a brief discussion. According to Priestley and Scott (1936, p. 160-161), within the growing organs the procambial cells elongate and divide transversely. Later "the dominant plane of cell division changes to the tangential longitudinal." With the advent of such divisions the meristem ceases to be a procambium and becomes a cambium. Kundu (1942, p. 98) similarly interprets the early stages of vascular differentiation in *Cannabis*: the procambial cells (Kundu uses "prodesmogen" instead of "procambium") "are elongated meristematic cells which divide most frequently by transverse walls, but occasionally also by longitudinal walls in any plane." When tangential longitudinal divisions begin to predominate, cambial activity and secondary growth set in. The present writer (Esau, 1943b) has previously emphasized that the mere occurrence or lack of radial seriation in the vascular meristem is an insufficient criterion for a separation of procambium from cambium, or primary from secondary growth; but the matter of occurrence of longitudinal divisions in the early stages of procambial differentiation has not been enough commented upon. Articles paying special attention to early vascularization (e.g., Sharman, 1942, fig. 10; Esau, 1943a, 1943c) prove that

in a given organ the procambium is initiated by a series of longitudinal divisions. The present study shows that, contrary to Priestley and Scott (1936), in *Helianthus* also the early stages of vascular differentiation are characterized by longitudinal divisions. More or less early (depending on the kind of plant) the longitudinal procambial divisions tend to become oriented tangentially and the procambium gives rise to cells in radial series like the cambium.

SUMMARY

The first sieve tubes of the apical portions of *Helianthus* and *Sambucus* shoots usually differentiate acropetally from the axis into the leaf in continuity with the sieve tubes of the older traces. The continuous acropetal differentiation may be followed, however, by a discontinuous maturation of

the elements composing a given sieve tube: at certain stages one or more series of immature elements may alternate with series of mature elements.

The earliest xylem is initiated in the upper parts of the shoot and differentiates upward toward the apices of the leaves and downward into the axis where ultimately it becomes connected with the xylem of other traces.

In a given leaf the first phloem elements mature before the first xylem elements; in other words, the first phloem differentiates one or more plastochrones in advance of the first xylem. The vascular elements appear first in the median and later in the lateral bundle of a given leaf.

COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

LITERATURE CITED

- BOND, T. E. T. 1942. Studies in the vegetative growth and anatomy of the tea plant (*Camelia thea* Link.) with special reference to the phloem. *Annals Botany* 6: 607-630.
- CRAFTS, A. S. 1943a. Vascular differentiation in the shoot apex of *Sequoia sempervirens*. *Amer. Jour. Bot.* 30: 110-121.
- . 1943b. Vascular differentiation in the shoot apices of ten coniferous species. *Amer. Jour. Bot.* 30: 382-393.
- ENGARD, C. J. 1944. Organogenesis in *Rubus*. *Univ. of Hawaii Res. Publ.* 21, 234 p.
- ESAU, K. 1938. Ontogeny and structure of the phloem of tobacco. *Hilgardia* 11: 343-424.
- . 1942. Vascular differentiation in the vegetative shoot of *Linum*. I. The procambium. *Amer. Jour. Bot.* 29: 738-747.
- . 1943a. Vascular differentiation in the vegetative shoot of *Linum*. II. The first phloem and xylem. *Amer. Jour. Bot.* 30: 248-255.
- . 1943b. Origin and development of primary vascular tissues in seed plants. *Bot. Rev.* 9: 125-206.
- . 1943c. Ontogeny of the vascular bundle in *Zea Mays*. *Hilgardia* 15: 327-368.
- KUNDU, B. C. 1942. The anatomy of two Indian fibre plants, *Cannabis* and *Corchorus* with special reference to fibre distribution and development. *Jour. Indian Bot. Soc.* 21: 93-128.
- PRIESTLEY, J. H., AND L. I. SCOTT. 1936. The vascular anatomy of *Helianthus annuus* L. *Proc. Leeds Phil. and Lit. Soc.* 3: 159-173.
- , AND K. M. MATTINSON. 1937. Dicotyledon phyllotaxis from the standpoint of development. *Proc. Leeds Phil. and Lit. Soc.* 3: 380-388.
- REEVE, R. M. 1942. Structure and growth of the vegetative shoot apex of *Garrya elliptica* Dougl. *Amer. Jour. Bot.* 29: 697-711.
- SHARMAN, B. C. 1942. Developmental anatomy of the shoot of *Zea Mays* L. *Annals Botany* 6: 246-282.

BRAZILIAN CHYTRIDS. V. NOWAKOWSKIELLA MACROSPORA N. SP., AND OTHER POLYCENTRIC SPECIES¹

John S. Karling

IN A previous paper on Brazilian chytrids collected in the Amazon Valley, the author (1944a) reported the occurrence of *Nowakowskiella elegans*, *N. ramosa*, and *N. profusa* and described two new species, *N. elongata* and *N. granulata* from Brazil. Since that time a sixth species of the same genus has been isolated from decayed vegetable debris in water and moist soil samples collected in Acre Territory. This species has the same general appearance, structure, and type of development as the other known members of *Nowakowskiella* but differs markedly from them in the large size of its zoospores. This is its primary distinguishing characteristic, and because the zoospores surpass in size those of all other species of this genus, the writer regards this fungus

as a new species and proposes the name *N. macrospora* for it.

NOWAKOWSKIELLA macrospora n. sp.—Fungus saprophyticus. Rhizomycelio hyalino, profuso, copiose ramoso, partibus tenuibus 2-6 μ diam., incrementis pluribus non septatis, ovalibus, 8-16 \times 12-15 μ , fusiformibus, 5-7 \times 12-15 μ , elongatis aut irregularibus. Sporangii terminalibus aut intercalariis, hyalinis, apophysatis, sphaericis (14-40 μ), ovalibus (18-25 \times 23-30 μ), pyriformibus (8-30 \times 18-55 μ), aut elongatis (10-20 \times 30-60 μ); operculo ovalibus aut sphaericis, 5-8 μ . Zoosporis sphaericis, 10-12 μ , unico globulo refringenti conspicuo, 3-5 μ diam. Sporis perdurantibus sphaericis (12-22 μ), ovalibus (15-18 \times 20-25 μ), levibus; germinantibus ut prosporangii parte interiori emer-

¹ Received for publication September 9, 1940.

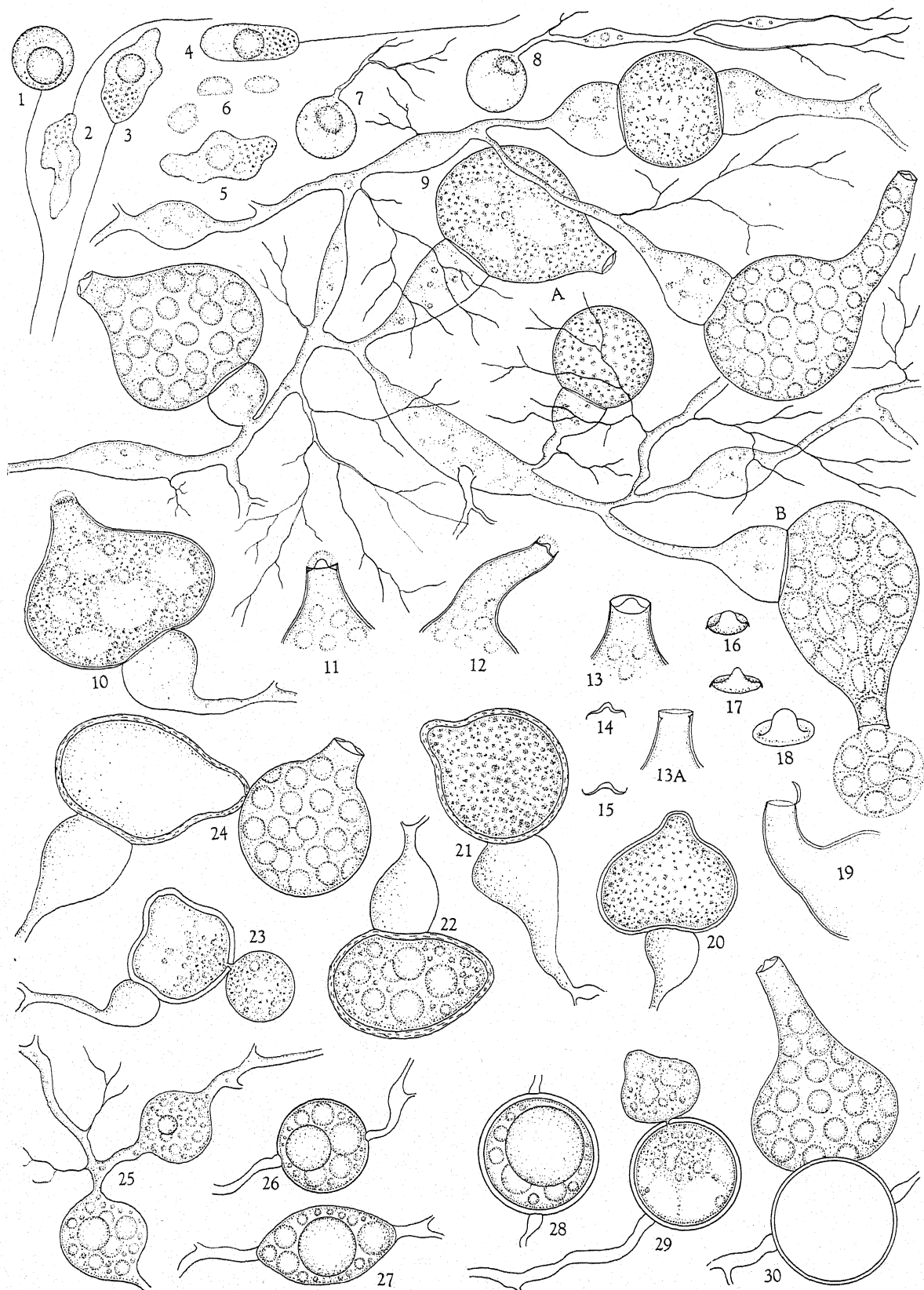


Fig. 1-30. *Nowakowskiella macrospora*.—Fig. 1. Zoospore during swimming stage. $\times 1250$.—Fig. 2-4. Amoeboid and creeping zoospores. $\times 1250$.—Fig. 5. Amoeboid zoospore after disappearance of flagellum. $\times 1250$.—Fig. 6. Varia-

gente ut zoosporangio membrano tenui ad superficiem sporae.

Rhizomycelium profuse, richly-branched, fairly coarse, tenuous portions 2–6 μ in diameter; spindle organs nonseptate, oval (8–16 \times 12–15 μ), broadly spindle-shaped (5–7 \times 12–15 μ), elongate and fusiform (6–10 \times 15–30 μ), or slightly irregular. Sporangia terminal or intercalary, hyaline, smooth, usually apophysate, often slightly flattened and elongated transversely to apophysis, spherical (14–40 μ), oval (18–25 \times 23–30 μ), pyriform (8–30 \times 18–55 μ), or elongate (10–20 \times 30–60 μ), often with an elongate neck, 5–8 \times 20–60 μ ; apophysis oval or nearly spherical, 8–20 μ in diameter, oblong, clavate or elongate; operculum usually slightly sunken, often apiculate and somewhat hat-shaped, 5–8 μ in diameter; sporangia sometimes becoming brown, thick-walled and dormant in old cultures, functioning as sporangia or prosperangia in germination. Zoospores slowly oozing out and forming a globular mass at exit orifice, spherical, 10–12 μ , with a large (3–5 μ), somewhat disc-shaped refractive globule, and numerous minute granules at posterior end; flagellum 38–42 μ long. Resting spores spherical (12–22 μ), oval (15–18 \times 20–25 μ), with a large refractive globule surrounded by numerous smaller ones, wall smooth, 1.5–2 μ thick, faintly yellowish-brown in color; functioning as prosperangia in germination.

Saprophytic in decayed vegetable debris, Boca do Acre, Acre Territory, Brazil.

Inasmuch as *N. macrospora* grows and develops in the same manner as other known species of this genus, it is not necessary to describe these processes again. In this relation, it will be sufficient to note that the germinating zoospores produce one or more germ tubes (fig. 9) which elongate, branch, and form intercalary swellings and rhizoids, and finally develop into the rhizomycelium (fig. 10). A portion of a mature thallus with variously-shaped non-septate swellings, rhizoids and sporangia is shown in figure 11. Of all known species, *N. macrospora* resembles *N. elegans* and *N. ramosa* most closely, particularly in relation to the apophysate sporangia. However, its sporangia are more often appressed and elongated at right angles to the apophysis (fig. 11A, 11B) than those of the former species. Furthermore, its intercalary swellings are usually more fusiform and elongate than in *N. elegans*.

The chief outstanding difference, as was noted above, is the large size of the zoospores (fig. 1–5).

These vary from 10 to 12 μ in diameter and surpass in size those of *N. ramosa* by several microns. Their most conspicuous structure is a large highly refringent globule which varies from 3 to 5 μ in diameter. It is usually somewhat flattened, disc-shaped, or slightly angular (fig. 6), and plastic in consistency, so that when the zoospore elongates and passes through a narrow space in the debris it changes markedly in shape (fig. 2). Occasionally, two large globules may be present (fig. 5) in one spore. In addition to the globule, the zoospores contain numerous small granules which lie near the posterior end. In emerging from the sporangium the zoospores ooze out quite slowly, so that three minutes may often elapse before the sporangium is emptied. The spores first form a globular mass at the exit orifice as in other species of *Nowakowskiella* (fig. 9B) and then separate slowly and swim away. In contrast to those of smaller chytrids, the zoospores seem to swim quite slowly, but this impression of slowness is primarily due to their large size. They may come to rest, and then creep around as small amoebae (fig. 2, 3) or short rods (fig. 4) with the flagellum trailing behind. At the close of the motile period the flagellum disappears, and the spore may move around slowly like an amoeba (fig. 5) before rounding up and germinating (fig. 7).

Another distinguishing characteristic of *N. macrospora* is the shape of the opercula and their method of development. In this process the tip of the exit papilla softens (fig. 10) and forms a low hemispherical plug of opaque material which extends a short distance into the tube. While these changes are taking place at the tip, the operculum is formed beneath the opaque material (fig. 11, 12) in much the same manner as Miss Johanson (1944) and the author (1944a, 1944b) have described for *Karlingia rosea*, *Nowakowskiella granulata*, and *Nephrochytium amazonensis*, respectively. In the present species, however, the amount of opaque material at the tip is smaller and less conspicuous. As the sporangia mature, this material usually disappears so that the operculum appears as a circular lid down in the papilla or tube (fig. 13). It is usually apiculate, thick in the center, and thin at the periphery. In longitudinal section it has the appearance shown in figure 14 as it lies free in the water outside the sporangium. The thin peripheral border may later turn up (fig. 15), so that in total surface view the opercula often look like small hats with upturned brims (fig. 16–18). In empty sporangia the region

tions of the shape of the refractive globule. \times 1250.—Fig. 7, 8. Germinated spores. \times 1250.—Fig. 9. Portion of rhizomycelium. \times 800.—Fig. 10. Softening of tip of exit papilla. \times 800.—Fig. 11, 12. Formation of operculum under mass of opaque material. \times 1000.—Fig. 13. Short exit tube in which opaque material has disappeared. \times 1250.—Fig. 13A. Longitudinal section of empty neck showing region where operculum was attached. \times 1000.—Fig. 14. Longitudinal section of operculum shortly after being pushed out of neck. \times 1000.—Fig. 15. Longitudinal section of operculum with thin upturned periphery. \times 1000.—Fig. 16–18. Hat-shaped opercula in total surface view. \times 1250.—Fig. 19. Attached operculum. \times 1000.—Fig. 20–22. Development and encystment of dormant sporangia. \times 1000.—Fig. 23, 24. Germination of dormant sporangia. \times 1000.—Fig. 25. Two incipient resting spores developing from intercalary enlargements. \times 1000.—Fig. 26, 27. Later developmental stages. \times 1000.—Fig. 28. Maturing resting spore. \times 1000.—Fig. 29, 30. Germination of resting spore. \times 1000.

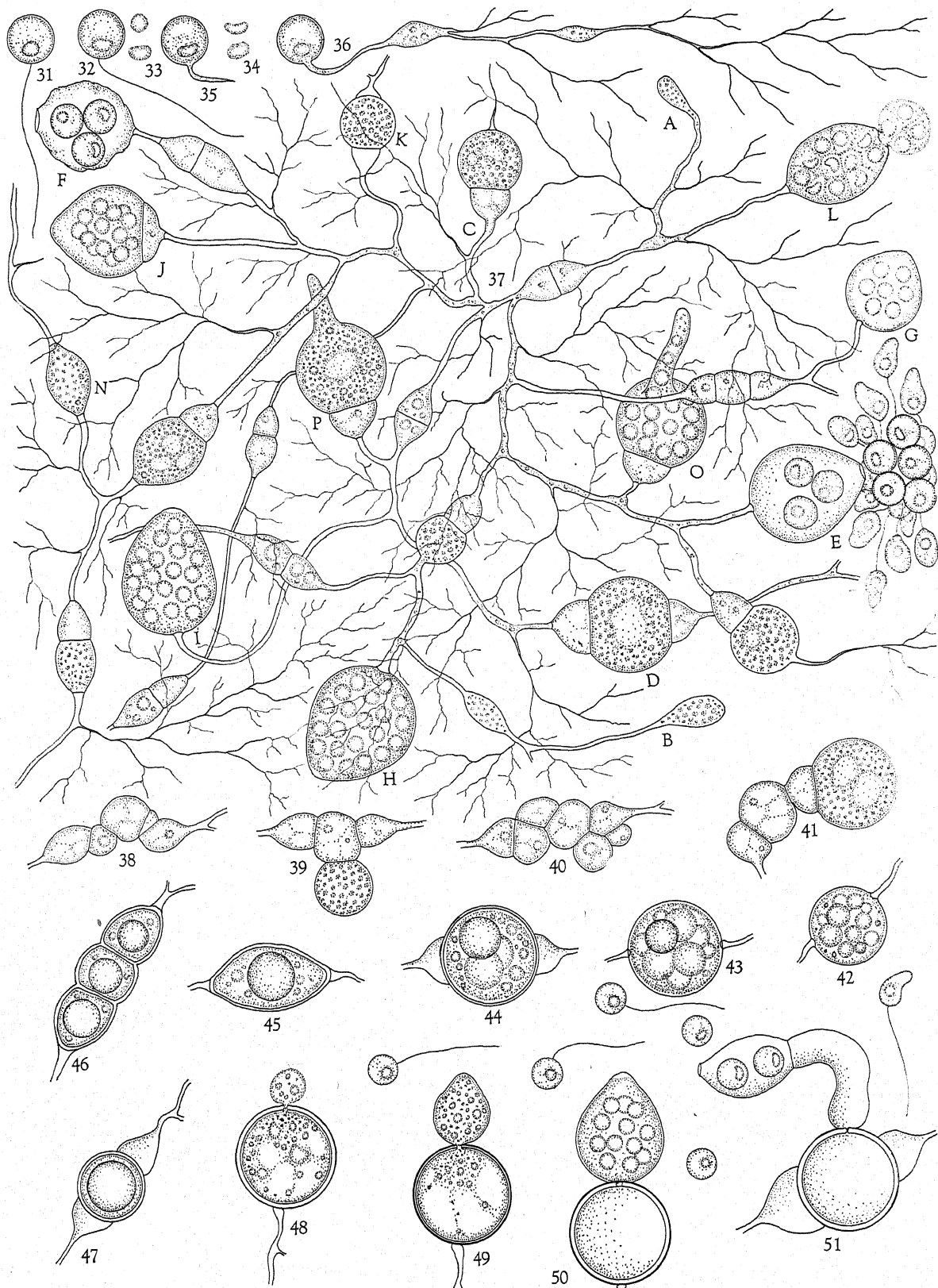


Fig. 31-51. *Cladochytrium elegans*.—Fig. 31, 32. Zoospores. $\times 1800$.—Fig. 33, 34. Variations in shape of refractive globule. $\times 1800$.—Fig. 35, 36. Germinating spores. $\times 1800$.—Fig. 37. Portion of the rhizomycelium. $\times 1200$.—Fig. 38-

of attachment of the operculum to the sporangium wall is often visible as a circular ridge inside the papilla or neck (fig. 13A).

It is to be noted here that not all of the opercula of *N. macrospora* are formed in the manner described above. Sometimes the tip of the exit papilla remains unchanged as the sporangium matures, with the result that an operculum develops at the surface as in *N. elegans*, *N. ramosa*, and other operculate species. Then, as the zoospores emerge, it is pushed off or remains attached at one side, as shown in figure 19.

As was noted in the diagnosis above, sporangia in old cultures may become light brown in color, thick-walled, and go into a dormant phase (fig. 20–22). Occasionally the protoplasm undergoes marked changes and forms numerous large refractive globules (fig. 22) like those present in the more usual type of resting spores. When conditions become favorable, the dormant sporangia germinate, and in so doing their content may either undergo cleavage directly into zoospores which escape through a papilla or tube, or it may emerge slowly (fig. 23) and form a thin-walled zoosporangium on the surface as shown in figure 24. In the latter event the dormant sporangium functions as a prosperangium and does not differ in this respect from the so-called true resting spores. Accordingly, such sporangia are regarded by the writer as a second type of resting spore in *N. macrospora*. The more common resting spores, however, are fundamentally similar to those of other *Nowakowskiella* species, and usually develop from the intercalary swellings in the rhizomycelium. Swellings which are to become resting spores are easily recognizable by the accumulated refractive globules (fig. 25). As they grow in size they are delimited from the remainder of the rhizomycelium by cross septa and become thick-walled. At the same time the refractive globules coalesce into increasingly larger ones (fig. 26, 27), so that at maturity the resting spore usually contains a large central globule surrounded by several smaller ones (fig. 28). After two months or more of dormancy, the spores germinate if environmental conditions are favorable. In this process they function as prosperangia and give rise to a thin-walled zoosporangium on their surface, as shown in figures 29 and 30.

SPECIES OF CLADOCHYTRIUM.—*Cladochytrium tenue*.—This is the type species of the genus which Nowakowski found in decaying tissues of various aquatic plants in Germany in 1876. Since that time it has been reported from France (de Wildeman, 1895), Romania (Constantineanu, 1901), Denmark (Petersen, 1909, 1910), and the United States (Karling, 1941; Sparrow, 1943), but it is not certain that

all of these records in the literature relate to *C. tenue*. It is not improbable that some of them may relate to *C. crassum* Hillegas (1941) and *C. hyalinum* Berdan (1941), hyaline species which appear to occur more commonly and are widely distributed (Karling, 1942). Neither de Wildeman nor Constantineanu saw the discharge, structure, and size of zoospores, so that it is not certain which species they had at hand. Petersen likewise was not certain that the species which he found was *C. tenue*.

The writer found what he interprets to be *C. tenue* in decaying vegetable debris from water and moist soil collected in northwestern Matto Grosso near the border of Bolivia, at São Carlos, Matto Grosso, Rio Madeira at Porto Velho, Amazonas, and in Igarape Raimundo at Manaus, Amazonas. This fungus thus appears to be widely distributed as a saprophyte in the Amazon Valley. It was isolated from the water and soil cultures on bits of onion skin and corn leaves and transferred to corn meal agar on which it grew fairly well. On synthetic agar media its growth appears to be sparse because of the fineness of the rhizomycelium and resembles that of *C. replicatum* except for its hyaline color.

Inasmuch as the life cycle of *C. tenue* is only partly known and has not been adequately illustrated, a brief description with figures is presented here of its development and structure. The zoospores (fig. 31, 32) are spherical, 4.5 to 5.5 μ , with a flagellum 25–28 μ long, and contain a conspicuous hyaline refractive globule which varies from 2 to 2.5 μ in diameter and is usually somewhat crescentic or disc-shaped (fig. 33, 34). The zoospores germinate with one or more germ tubes (fig. 35, 36), as described by Nowakowski and Sparrow, and give rise to the polycentric rhizomycelium, a portion of which is shown in figure 37. The tenuous portions of the rhizomycelium are usually very fine, 1.5 to 3.5 μ in diameter, and bear numerous rhizoids like those of *C. replicatum*. The intercalary swellings or spindle organs are nearly always septate, fusiform, elongate (5–8 \times 10–17 μ), oval (6–8 \times 9–12 μ), or oblong. In rapidly growing cultures the majority are uniseptate and slightly constricted at the septum, but they may occasionally become bi-, tri- and multiseptate, like those described by the author (1935, 1937) for *C. replicatum*. In old cultures they sometimes proliferate and form masses of cells (fig. 38, 40). Similar proliferation may also occur in relation to sporangium and resting spore formation in old cultures (fig. 39, 41), but this is not so common an occurrence as in *C. hyalinum*. The cells of the spindle organs usually contain one or more refractive globules as described by Nowakowski, but if they are to give

41. Proliferation of spindle organs. \times 1300.—Fig. 42, 43. Incipient resting spores developing from intercalary swellings. \times 1600.—Fig. 44. Apophysate resting spore which has developed from a three-celled spindle organ. \times 1400.—Fig. 45. Mature oval resting spore. \times 1600.—Fig. 46. A spindle organ, the three cells of which have developed into resting spores. \times 1600.—Fig. 47. Small mature spherical apophysate resting spore. \times 1600.—Fig. 48, 49. Germinating resting spores. \times 1600.—Fig. 50, 51. Germinated spores with pyriform and elongate septate sporangia, respectively. \times 1600.

rise to sporangia, their content is dense and coarsely granular (fig. 37C, K, N).

The majority of sporangia in onion skin and corn leaf cultures are terminal and develop extramatrically on slender filaments 1.5–2 μ in diameter (fig. 37E, G, H, I, J). As they stand out from the surface of the substratum they have the appearance of oval, sub-spherical or slightly citriform hyaline blisters filled with glistening spheres or globules. These terminal sporangia begin as swellings at the tips of branches (fig. 37A, B), the contents of which are at first granular and often vacuolate. As they attain maturity, the granular appearance of the protoplasm disappears and is replaced by numerous refractive globules which appear to be suspended in a clear ground substance. Sparrow reported that he did not find the globules aggregated toward the center of the sporangia as in *C. replicatum*, but in the Brazilian material of *C. tenue* it is a common occurrence (fig. 37G, J). From careful study of developing sporangia of both species the present writer concludes that aggregation of the globules is a developmental phase and not a specific character. Later, the globules become more or less evenly distributed throughout the sporangia in both species.

The terminal extramatrical sporangia of *C. tenue* rarely form tubes for discharge of the zoospores and also lack prominent exit papillae. Instead, the tip of the sporangium is only slightly raised and pointed, and underneath lies a small clear biconvex area. The tip deliquesces when the sporangia are mature, and the zoospores emerge to form a globular cluster at the exit orifice (fig. 37L) and soon disperse (fig. 37E).

The sporangia may also be intercalary and develop directly as swellings in the rhizomycelium (fig. 37N) or from a cell of the spindle organ (fig. 37D, O, P). In either event, the sporangial rudiments may be recognized very early by their granular content and by the presence of small vacuoles. Their further development and maturation are similar to those of the terminal sporangia described above. When the sporangia develop intramatrically, an exit tube is formed for the discharge of the zoospores to the outside of the substratum (fig. 37 O, P). As was noted earlier, the majority of sporangia are broadly oval or slightly citriform (8–18 $\mu \times$ 10–22 μ), with a broader basal end, or spherical (8–30 μ), or somewhat pyriform. Nowakowski reported that he found sporangia up to 66 μ in diameter, but none above 30 μ has been observed in the material from Brazil. On numerous occasions minute sporangia with only four zoospores were found. The wall of the sporangium is very thin, and shortly after the zoospores are discharged it becomes wrinkled and collapses (fig. 37F). However, if the sporangium proliferates and forms a secondary sporangium inside, the wall becomes distended again, but it usually does not lose its wrinkled appearance.

Up to the present time, resting spores have been unknown in *C. tenue*, but the author found them in abundance in old onion skin cultures of the Brazilian material. They are spherical, (8–16 μ), oval (10–12 \times 14–16 μ), or broadly fusiform, hyaline and smooth (fig. 44, 45, 46). As described above for *N. macrospora*, they usually originate from intercalary swellings or cells of the septate spindle organs. Cells which are to become resting spores accumulate a large amount of refractive substance in the form of globules (fig. 42), enlarge (fig. 43), and become invested with a fairly thick hyaline wall (fig. 44). At the same time the globules coalesce so that at maturity the resting spore usually contains a single globule which occupies the large part of its lumen (fig. 47). Occasionally several smaller ones may also be present (fig. 45, 46), and the remainder of the protoplasm is finely granular in appearance. Sometimes all of the cells of a septate spindle organ develop into resting spores, as shown in figure 46. When only the central cell develops, the resting spore is apophysate on both sides (fig. 44, 47, 51). After a dormant period of two or more months, the spores germinate if conditions are favorable. In so doing they function as prosperangia and give rise to thin-walled evanescent sporangia (fig. 48–51) on their surfaces. These sporangia are usually similar in size and shape to those described above, but occasionally they may become elongate and septate (fig. 51). The discovery of resting spores and their germination thus completes the life cycle of *C. tenue*, which is fundamentally similar to that of other species of *Cladochytrium*.

Cladochytrium crassum.—This species was isolated on bits of onion skin and corn leaves from water collected at São Carlos, Matto Grosso. The structure and development of the Brazilian material are so similar to those described by Hillegas (1941) that it is unnecessary to describe them further. It may be noted, however, that Hillegas failed to secure germination of the resting spores in the American material. The resting spores from Brazil, on the other hand, germinated readily in laboratory cultures after a dormant period of seven weeks. In this process they behaved like the resting spore of *N. profusa* (Karling, 1941, 1944). Some of them functioned as sporangia and formed zoospores directly which emerged from a thin-walled exit tube. In others the content slowly emerged through a pore in the wall and developed into a sporangium of the type described above for *C. tenue*.

Cladochytrium hyalinum.—This species occurred in abundance in onion skin and corn leaves which had been added to water cultures collected in northwestern Matto Grosso near the Bolivian boundary, and at Porto Velho, Amazonas. No fundamental variations and differences in development, structure, and germination of the resting spores were observed in the Brazilian specimens, and it is not necessary to amplify the detailed and adequate description given by Miss Berdan (1941).

Cladochytrium replicatum.—This species is widely distributed in the Amazon Valley and has been collected in various parts of Acre Territory, Matto Grosso, Amazonas, and Ceara. It occurs in most small streams, ponds, lagoons, and lakes as a saprophyte in decayed vegetable debris. No essential differences between the Brazilian and North American material were observed.

SUMMARY

Nowakowskiella macrospora n. sp. occurred as a saprophyte in decayed leaf mold collected in Acre Territory, Brazil. It differs primarily from other known species of this genus by its large zoospores which vary from 10 to 12 μ in diameter. In development, thallus structure, and organization it is similar to other species of *Nowakowskiella*. Dormant

thick-walled sporangia are sometimes formed which may give rise directly to zoospores or function as prosperangia in germination. Resting spores are formed in old cultures; in germinating, their contents emerge and form thin-walled sporangia on their surfaces. The discovery of this species raises the number of species of *Nowakowskiella* to eight, all of which have been found in Brazil except *N. hemisphaerospora* (Shanor, 1942) and *N. delicata* (Whiffen, 1943).

In addition to these species, four other polycentric cladochytriaceous chytrids were isolated from water cultures: *Cladochytrium tenue*, *C. crassum*, *C. hyalinum*, and *C. replicatum*.

DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY,
NEW YORK, NEW YORK

LITERATURE CITED

- BERDAN, H. B. 1941. A developmental study of three saprophytic chytrids. I. *Cladochytrium hyalinum*. Amer. Jour. Bot. 28: 422-428.
- CONSTANTINEANU, M. J. C. 1901. Contributions a la flore mycologique de la Roumaine. Rev. Gen. Bot. 13: 369-389.
- HILLEGAS, A. B. 1941. Observations on a new species of *Cladochytrium*. Mycologia 33: 618-632.
- JOHANSON, A. 1944. An endo-operculate chytridiaceous fungus, *Karlingia rosea*, n. gen. Amer. Jour. Bot. 31: 397-404.
- KARLING, J. S. 1931. Studies in the Chytridiales. VI. The occurrence and life history of a new species of *Cladochytrium* in cells of *Eriocaulon septangulare*. Amer. Jour. Bot. 18: 526-577.
- . 1935. A further study of *Cladochytrium replicatum* with special reference to its distribution, host range, and culture on artificial media. Amer. Jour. Bot. 22: 439-452.
- . 1937. The cytology of the Chytridiales with special reference to *Cladochytrium replicatum*. Mem. Torrey Bot. Club 19: 1-92.
- . 1941. *Cylindrochytridium Johnstonii* gen. nov. et sp. nov., and *Nowakowskiella profusum* sp. nov. Bull. Torrey Bot. Club 68: 381-387.
- . 1942. A new chytrid with giant zoospores: *Septochytrium macrosporum* sp. nov. Amer. Jour. Bot. 29: 616-622.
- . 1944a. Brazilian chytrids. I. Species of *Nowakowskiella*. Bull. Torrey Bot. Club 71: 374-389.
- . 1944b. Brazilian chytrids. III. *Nephrochytrium amazonensis*. Mycologia 36: 351-357.
- NOWAKOWSKI, L. 1876. Beitrag zur Kenntniss der Chytridiaceen. Cohn's Beitr. Biol. Pflanzen 2: 73-100.
- PETERSEN, H. E. 1909. Studier over Ferskvands-Phycomyceten. Bot. Tidsskr. 29 (4): 345-440.
- . 1910. An account of Danish freshwater Phycomycetes with biological and systematical remarks. Ann. mycol. 8: 494-560.
- SHANOR, L. 1942. A new fungus belonging to the Cladochytriaceae. Amer. Jour. Bot. 29: 174-179.
- SPARROW, F. K. 1943. The aquatic Phycomycetes. Univ. of Michigan Press, Ann Arbor.
- WHIFFEN, A. J. 1943. New species of *Nowakowskiella* and *Blastocladia*. Jour. Elisha Mitchell Sci. Soc. 59: 37-43.
- WILDEMAN, DE, E. 1895. Notes mycologiques. XV. Mem. Soc. Belge. Micro. 19: 88-117.

GROWTH AND DIFFERENTIATION IN THE ROOT TIP OF PHLEUM PRATENSE¹

Richard H. Goodwin and William Stepka

MORE DETAILED information than is at present available in the literature is needed concerning the nature of growth and differentiation of the apical meristems. Roots, which are not obscured or complicated by foliar emergences, should be the most favorable subjects for such studies. Modern text-

In 1939, Sinnott pointed out the suitability of certain species of small-seeded grasses for studies of the growing root tip. In these species the transparency of the fine roots and the rudimentary character of the root caps permit unobscured microscopic observation of the surface of the living meristems. Brumfield (1942) has made some interesting experimental studies on one of these species, *Phleum pratense* L., in which rates of elongation of roots at various distances from the root apex were measured.

In this paper methods are developed for determining rate of elongation of a root, cell elongation, rate of transverse cell division, and rate of increase in cell wall area, each expressed as a function of distance from the root apex. These methods are then applied to roots of *Phleum pratense*. The resulting data give a graphic picture of the above-mentioned developmental processes which are taking place within the growing region of a root and of the complex interrelations between them. Some further observations on the differentiation of vascular elements are correlated with these findings.

THEORETICAL METHODS.—Rate of elongation.—Seedlings of grasses such as *Phleum pratense* may be grown in vertical culture chambers so constructed as to allow examination of the root meristems under high magnifications with a compound microscope. It is possible to measure the amount of growth occurring during short time intervals between the root apex and characteristically-shaped epidermal marker cells. When such growth increments in unit time (r) are plotted against the initial distance (x) of the marker cells from the root apex, sigmoid curves, representing the rates of displacement of x from the root apex, are obtained (fig. 2). It should be pointed out that the time interval must be sufficiently short to prevent undue displacement of the marker cell from its original position, x units of distance from the root apex. The first derivatives of these sigmoid curves, dr/dx (the slope of the tangent to the curve at any point), give the rates of elongation of the roots as a function of distance from the tip (fig. 3).

Average length of epidermal cells.—The length (l) of epidermal cells at various distances from the root apex may be readily determined. Camera lucida drawings of the surface of actively-growing roots cannot be made with sufficient rapidity to serve for this purpose. If large numbers of cells are to be measured, it may be convenient to make such measurements upon roots killed in fixatives which do not appreciably distort the cell dimensions, although measurements from photographs of living roots should be preferable. The length of cells in the sub-epidermal layers may be studied from longitudinal sections. Figure 4 shows the average length of epidermal cells with their basal ends at x .

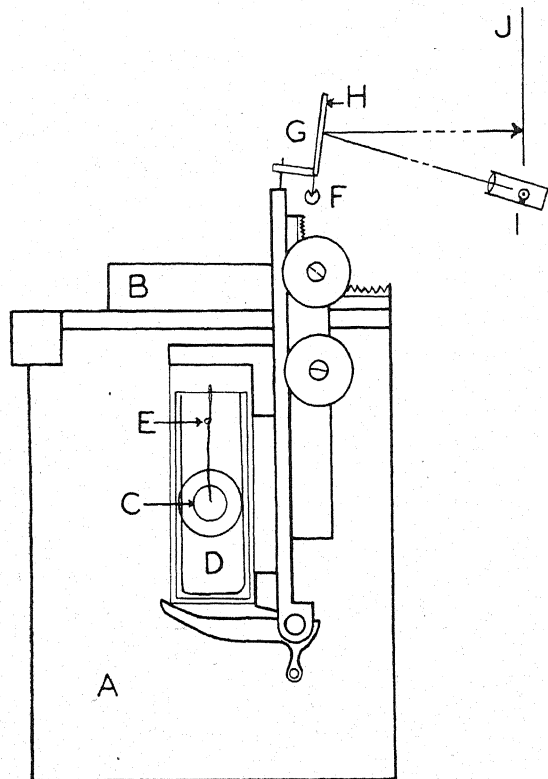


Fig. 1. Diagram of a device for recording growth increments.—A. Vertical microscope stage.—B. Mechanical stage.—C. Upper element of the substage condenser.—D. Glass culture chamber.—E. Seedling.—F. Fixed support.—G. Light lever.—H. Mirror surface.—I. Galvanometer lamp projecting the image of a cross hair.—J. A scale three meters long placed at a distance of 20 feet.

books usually figure the root tip in longitudinal section with various regions marked off: root cap, meristematic zone, zone of elongation, and zone of differentiation or maturation. Such descriptive demarcations fail to give quantitative information concerning the absolute or even the relative rates at which the various developmental processes, which are supposed to be occurring in these regions, are actually taking place. The nature of the transitions between one zone and the next are often completely ignored.

¹ Received for publication July 14, 1944.

Growth rate measurements were made by the junior author, who is now serving with the armed forces.

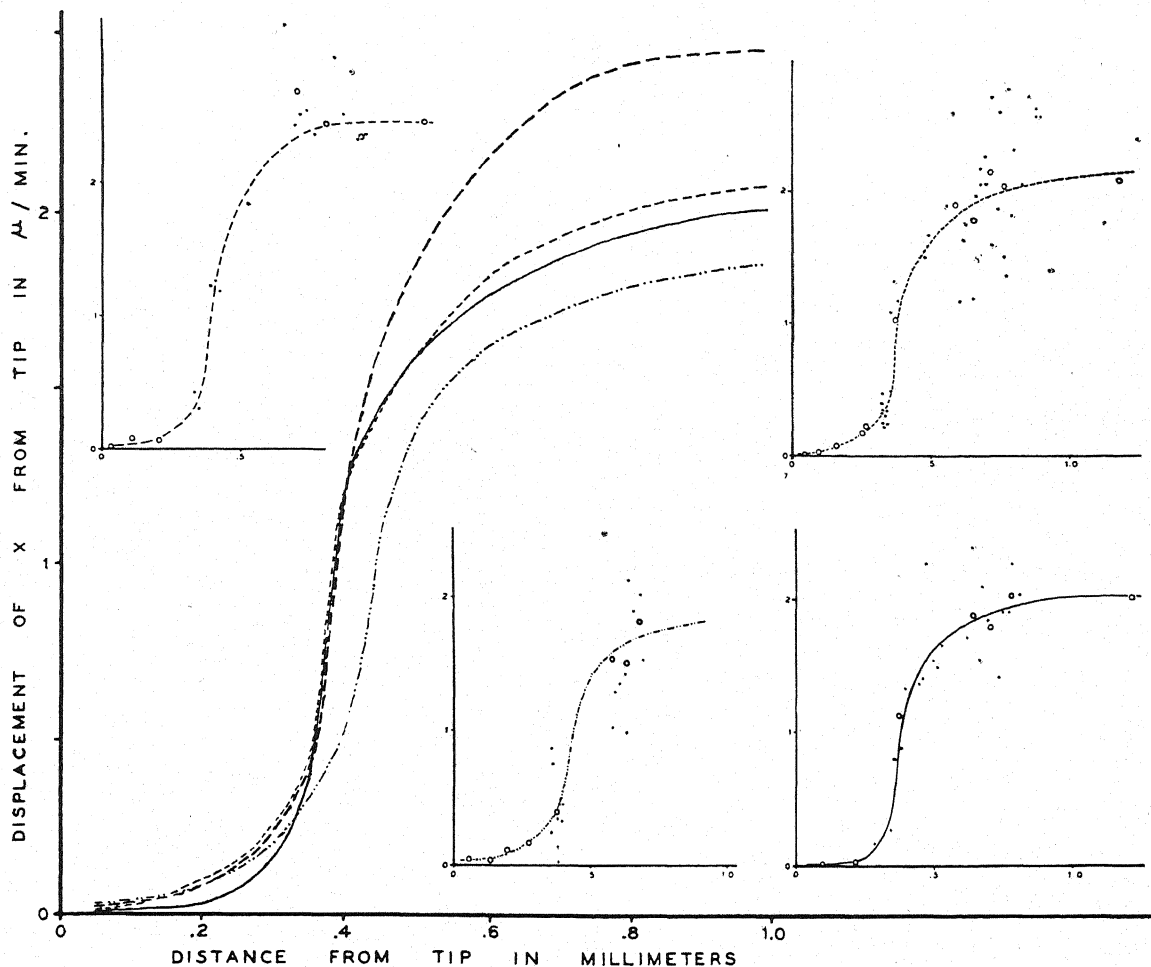


Fig. 2. The rate of displacement from the root apex of various points on the surface of the root plotted as a function of distance from the apex. The smaller graphs show the original data for four different roots. Open circles in the apical 300 μ of the root represent rates for time intervals greater than ten minutes; while further back they represent averages of the individual values shown for narrow zones of the root. The larger graph shows the smoothed curves superposed.

Rate of cell division in the epidermis.—There are several ways in which the frequency of cell division may be investigated. One line of attack would be a statistical study of the frequency of mitotic figures in cytological preparations (Wagner, 1930), but an accurate interpretation of data so obtained is most difficult. Actual counts may be made of the number of new cells formed among a given group of cells by comparing successive photographs taken at definite time intervals (Brumfield, 1942). If this method is employed, the time intervals should be very short to reduce the displacement from the root apex of the region studied. A third approach is presented below.²

The problem is to determine for a particular cell lineage of a root the frequency of cell divisions perpendicular to the long axis at various distances from the root apex. The following solution depends upon the assumption that the pattern of development, and, hence, average cell size at the different levels of the

root, remains essentially constant, at least for periods of several hours. Meristems which exhibit irregular or rhythmic mitotic behavior can not be used for an analysis of this sort.

Suppose we regard the root apex as a fixed point, from which the matured, basal portions of the root are being displaced by growth. Now select a point some arbitrary distance, x microns from the root apex. How often during growth would transverse cell walls be carried past this point? At the moment when a wall is just passing point x , the adjacent more apical wall is at a point $x - l$, where l is the average length of cells with their basal ends at x . This more apical wall will move toward x at a rate $1/2 (r_x + r_{x-l})$, where r is the rate of displacement of a point from the apex. Hence, it will take $2l/(r_x + r_{x-l})$ time units for this next wall to pass x . In that time, however, one new cell wall must be laid down between the root apex and x , otherwise the average length of cells at some point between the apex and x would have changed. In other words, one

²The authors are greatly indebted to Dr. Donald R. Charles for his assistance in developing this method.

new cell wall must be formed every $2l/(r_x + r_{x-1})$ minutes, or the rate of wall formation between the apex and x must be $(r_x + r_{x-1})/2l$ walls per minute. Employing the calculus, the first derivative of this last expression will give the rate of deposition of new transverse cell walls at any particular distance from the root apex. Thus

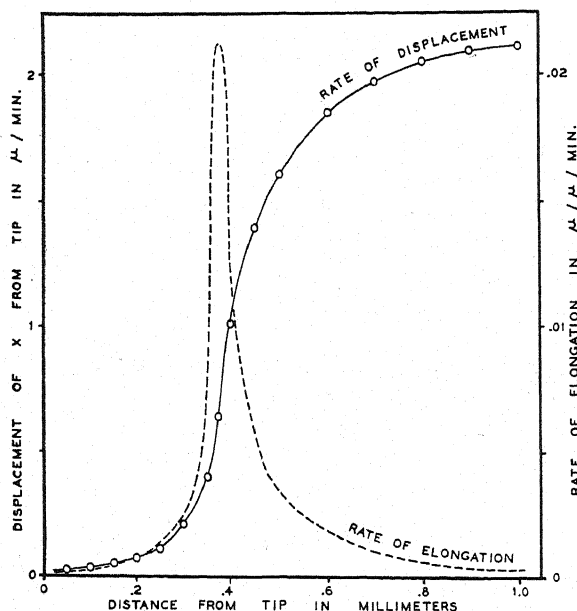


Fig. 3. Solid line: rate of displacement from the root apex of various points on the surface of the root, plotted as a function of distance from the apex. This curve is the average of the curves shown in figure 2. Broken line: rate of elongation of the root, plotted as a function of distance from the apex. This curve was obtained by averaging the first derivatives of the curves shown in figure 2.

$$\frac{1}{2} \frac{d}{dx} \left(\frac{r_x + r_{x-1}}{1} \right) = \frac{1}{2} \left[\frac{1 \left(\frac{dr_x}{dx} + \frac{dr_{x-1}}{dx} \right) - (r_x + r_{x-1}) \frac{dl}{dx}}{l^2} \right] = \frac{1}{2l} \left[\left(\frac{dr_x}{dx} - r_x \frac{dl}{l dx} \right) + \left(\frac{dr_{x-1}}{dx} - r_{x-1} \frac{dl}{l dx} \right) \right]$$

For practical purposes it may be sufficient to take

$$\frac{1}{1} \left[\frac{dr_x}{dx} - \frac{r_x}{1} \frac{dl}{dx} \right] \quad (1)$$

Two sets of data, which have already been discussed, are necessary: first, r , the rate of displacement from the apex of a series of points along the root (figs. 2 and 3); and second, l , the average length of cells the basal ends of which are at known distances from the root apex (fig. 4). The expressions dr_x/dx and dl/dx represent the first derivatives of (or the slopes of the tangents to) the curves shown in figures 2 and 4 respectively.

Rate of cell wall formation.—The primary body of roots, at least in the early developmental stages, may be thought of as consisting of a series of rows of cells which are dividing almost exclusively in the transverse plane. The increase in the area of transverse walls in a given row of cells at any particular level in the root will be directly proportional to the rate at which new cell walls are laid down in that row. If one assumes that the rate of cell division, determined for the epidermis, is essentially the same for all types of cells within the root,³ then the increase in area of transverse walls at a given level will be the product of the rate of cell division, formula (1), times the cross-sectional area of the root, $\pi D^2/4$, where D equals the diameter of the root.

$$\frac{\pi D^2}{4} \left[\frac{1}{1} \left(\frac{dr_x}{dx} - \frac{r_x}{1} \frac{dl}{dx} \right) \right] \quad (2)$$

Also, the increase in area of the longitudinal cell walls will be directly proportional to the rate of elongation. The amount of new longitudinal wall formed at any particular distance from the tip will be equal to the product of the rate of elongation and the length, L , of longitudinal wall intercepted by a cross section at that point (fig. 6).

$$\frac{dr_x}{dx} (L) \quad (3)$$

The amount of longitudinal wall, L , exposed in a cross section of a cylindrical, cellular structure (*i.e.*, the length of line seen in a cross section such as shown in fig. 6) may be estimated somewhat crudely by means of the following formula:

$$L = D (1.65 \sqrt{n} + 1.91) \quad (4)$$

where D equals the diameter of the cross section, and n equals the number of rows of cells intercepted by the cross section. This formula has been derived on the basis of the following assumptions: (1) that the cells all have the same diameter in cross section; (2) that they are hexagonal in shape (see Lewis, 1943); (3) that they are packed together into hexagonal aggregations; and (4) that the area of the circle passing through the centers of the hexagons comprising the corners of the hexagonal aggregate approximates the area of the hexagonal figure. For an error introduced by this last approximation see footnote 4. The derivation of formula (4) follows.

Let D equal the diameter of the circle, and s , the length of the sides of the individual hexagons. Then $\pi D^2/4$ equals the area of the circle and $3\sqrt{3}s^2/2$ equals the area of one of the hexagons. If $\pi D^2/4$ is set equal to $3\sqrt{3}s^2n/2$, where n equals the number of hexagons in the hexagonal aggregate,⁴ then:

³ For errors made by this assumption in the case of *Phleum* roots, see table 4.

⁴ The ratio $\frac{\pi D^2}{4} \div \frac{3\sqrt{3}}{2} s^2 n$ lies between 1.13 and 0.89 for values of n ranging from 37 to 631. For values of n larger than 631, the ratio asymptotically approaches the limit 0.827.

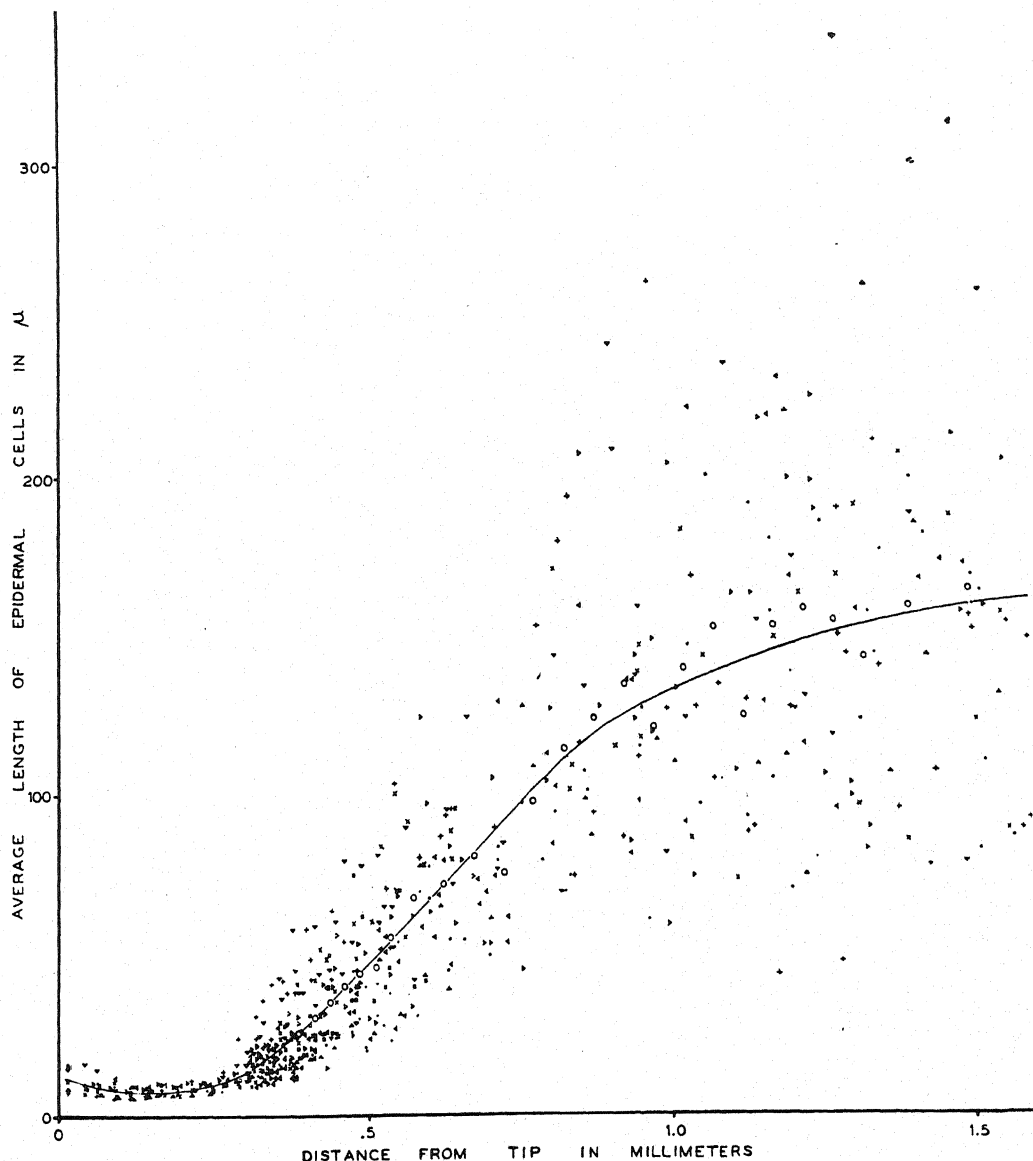


Fig. 4. The length of epidermal cells with their basal ends x mm. from the root apex, plotted against x . Individual measurements on eight different roots are shown, except within 300μ of the tip, where average values for each root are given at 25μ intervals. The circles represent averages for all values within narrow zones of the root, and the curve has been drawn by inspection through these points. The great variation in the size of the elongated cells is due in part to the fact that two cell types, which differ greatly in length, are included in the measurements. The data have not been corrected for shrinkage due to fixation.

$$s = \frac{D}{\sqrt{n}} \sqrt{\frac{\pi}{6\sqrt{3}}} = \frac{D}{\sqrt{n}} (.55)$$

The total number of hexagon faces in hexagonal aggregates of hexagons, shared faces being counted only once, is given by the expression:

$3n + \sqrt{12n - 3}$. Therefore,

$$L = D (.55) \left(\frac{3n}{\sqrt{n}} + \frac{\sqrt{12n - 3}}{\sqrt{n}} \right)$$

For values of

$$n > 10, \frac{\sqrt{12n - 3}}{\sqrt{n}} \cong 2\sqrt{3}, \text{ and } L = D(1.65\sqrt{n} + 1.91).$$

In general, this formula would be expected to apply best to those structures, such as meristematic root tips, in which the configuration of the cells most nearly approaches the foregoing assumptions. Since no one of these assumptions is strictly true, the accuracy of estimates of the amount of longitudinal wall derived by this method for any particular structure

TABLE 1. *A comparison of calculated and measured values for the length of wall intercepted by cross sections of roots of various species.*

Species	Root diameter in μ	No. cells in cross section	Length of wall calc. (μ)	Length of wall meas. (μ)	Per cent error	Source of cross section
	(=D)	(=n)	(=L)	(=L _m)	$\frac{L_m - L}{L_m} \times 100$	
<i>Sporobolus cryptandrus</i>	81.8	88	1423	1418	-0.4	Sinnott and Bloch, 1941. Figures 13 and 14
<i>Agrostis alba</i>	102.0	75	1652	1578	-4.7	
	128.0	91	2259	2300	1.8	Slide prepared by the senior author
<i>Phleum pratense</i>	134.2	109	2567	2536	-1.2	Slide prepared by the senior author
	138.7	94	2480	2488	0.3	Slide prepared by the senior author (see fig. 6)
<i>Nicotiana Tabacum</i> ...	143.0 ^a	120	2857	2859	0.1	Esau, 1941. Plate 9A
	147.4 ^a	156	3319	3316	-0.1	Esau, 1941. Plate 10A
<i>Daucus carota</i>	207.5	294	6264	6680	6.2	Esau, 1940. Plate 1A
	237.5	319	7454	7265	-2.6	Esau, 1940. Plate 2A
<i>Crepis capillaris</i>	239.9	338	7742	7692	-0.7	Slide borrowed from the collection at the University of Rochester
<i>Solidago rugosa</i>	254.0	894	13016	14424	9.8	Slide prepared by the senior author

^a Including endodermis and vascular cylinder only.

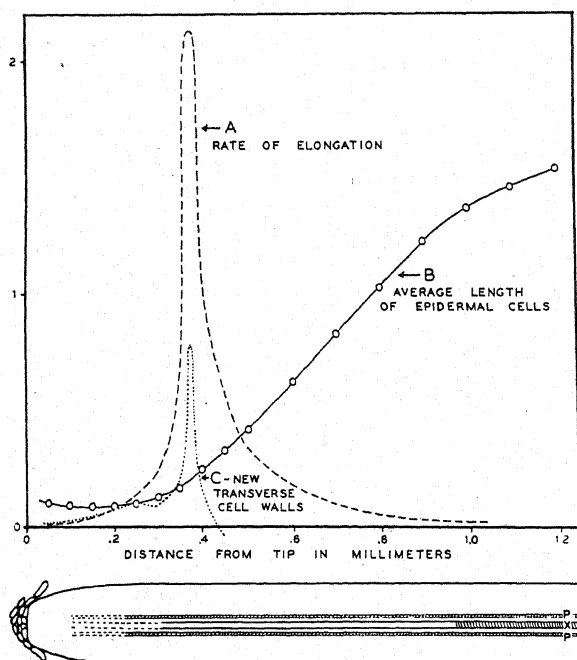


Fig. 5.—A. Rate of elongation of the root ($(\mu/\mu/\text{min.}) \times 10^2$), from figure 3.—B. Average length of epidermal cells with their bases at x ($\text{in } \mu \times 10^{-2}$). The original curve, shown in figure 4, has been corrected for an average shrinkage of 9.3 per cent.—C. The estimated rate of formation of new transverse cell walls in a single row of epidermal cells ($(\text{number of cells } / \mu/\text{min.}) \times 10^3$). Below the graph is a diagrammatic longitudinal section through the triarch root showing the levels at which the youngest vascular elements are differentiated. In the center is one of three rows of xylary cells (X). They may be recognized within approximately 100 μ of the root apex, become vacuolate at 300 μ and show characteristic secondary thickenings at 970 μ . Two of the three protophloem strands (P) are shown.

should be checked by actual measurements of photographs or camera lucida drawings. Such checks have been made for root tips belonging to seven different species. The results are summarized in table 1. It may be seen that the errors in the estimates do not exceed 6.2 per cent, with the exception of the *Solidago* root, in which the number of cells was rather large (see footnote 4). For *Phleum pratense* the estimates are within two per cent of the measured values.

Now substituting for L in formula (3), the rate of longitudinal wall formation will be:

$$D \left(\frac{dx}{dx} \right) (1.65 \sqrt{n} + 1.91)$$

The rate of total cell wall formation at any given distance from the tip may now be estimated. It will be equal to the sum of transverse wall formation, formula (2), plus longitudinal wall formation, formula (5).

EXPERIMENTAL METHODS AND RESULTS.—The species used in this investigation was Timothy grass, *Phleum pratense* L. The seed, obtained from Hart & Vick, Inc., of Rochester, New York, was from lot No. 1253, grown in Minnesota. Seeds were germinated on filter paper in petri dishes propped in a nearly vertical position. The filter paper was moistened with Knop's solution (0.8 g. of $\text{Ca}(\text{NO}_3)_2$, 0.2 g. of KNO_3 , 0.2 g. of KH_2PO_4 , 0.2 g. of MgSO_4 , and a trace of FePO_4 per liter of distilled water). The dishes were kept in the dark at $25^\circ \pm 0.5^\circ \text{C}$.

For growth-rate studies, mounts were made as follows. After germination, a young seedling was placed on a 24 x 60 mm. cover glass between two These may be recognized at 100 μ , and become mature at about 230 μ from the root apex.

closely-approximated strips of filter paper, and the root was covered with a strip of lens paper moistened with Knop's solution. The cover glasses thus prepared were placed in Coplin jars, with the lower portion of the strips of lens paper dipping into Knop's solution, until the primary roots had attained a length of about one centimeter. The cover slips were then used to form one side of a thin, elongated chamber, 75 x 25 x 1 mm., constructed of a glass slide to which had been cemented three strips of glass 1 mm. thick. The chamber was completed by sealing the cover glass—seedling on the inside—to the glass strips with a thin film of lubrisal, and a few drops of Knop's solution were introduced at the open end.

In order to obtain growth rate data, a chamber was attached in a vertical position to the mechanical stage of a horizontal microscope mounted on an optical bench (actually a Bausch & Lomb VHM projection apparatus). The light source was a 200 watt, 120 volt Mazda projection lamp used in conjunction with a 32 mm. water filter and a No. 3480 Corning (orange) filter. The substage diaphragm was stopped down to a point which would just allow accurate observation of the outlines of the epidermal

The position of the cross walls of a series of epidermal cells with characteristic shapes or other identifying features was followed at ten-minute intervals by adjusting the mechanical stage so that each of these cross walls would be brought successively into coincidence with a cross hair in the ocular. The position of the mechanical stage was then recorded on a vertical scale three meters long by means of a light lever, which reflected the image of a cross hair projected from a fixed galvanometer lamp. A diagram of the physical set-up is shown in figure 1. Successive recordings of six to eight cross walls were made at 30 second intervals. With this simple device a magnification of 1636 X was obtained, and individual measurements were made with a standard deviation of 1.05 μ .

All measurements were made with reference to the point of juncture between the base of the root cap and the apex of the root meristem. For practical purposes, this point, which could be accurately determined, will be referred to as the root apex. Points within 300 μ of the apex were displaced at such slow rates that time intervals of an hour or more could be used to calculate rates of displacement in this region. Further back, the ten-minute time intervals were used. Figure 2 shows the rates of displacement of points various distances from the root apex, for four different roots. Table 2 and figure 3 give the mean rates of displacement and also the mean rates of elongation for all four roots at various levels. It is to be regretted that these data are at present so scanty, but this phase of the work was interrupted by an exigency of the war.

It should be emphasized here that the growth rate will be greatly influenced by numerous environmental factors. A comparison of the over-all growth rate for the roots shown in figure 2 with that for roots grown under somewhat different conditions is made in table 3. The reasons for these differences in growth rate have not been determined, but illumination would not seem to be of much importance. Although slow, the growth rates in the experimental chambers were rather constant over a period of several hours. To what extent the developmental pattern may be affected by factors which influence the growth rate is not known.

For measurements of epidermal cell length, other roots, grown under conditions similar to those described for the growth-rate studies, were fixed in formalin-acetic-alcohol (5 cc. commercial formalin; 5 cc. glacial acetic acid; 100 cc. 50 per cent ethyl alcohol) and mounted in 70 per cent lactic acid. This treatment was found to cause a shrinkage of 9.3 per cent. Outlines of the epidermal cells were made with a camera lucida and the lengths of the cells were measured from these drawings. Figure 4 shows the lengths of epidermal cells plotted as a function of the distance of their basal walls from the root apex. The great variation in size of the elongated cells is due in part to the fact that two cell types, ordinary epidermal cells and trichoblasts, which differ greatly

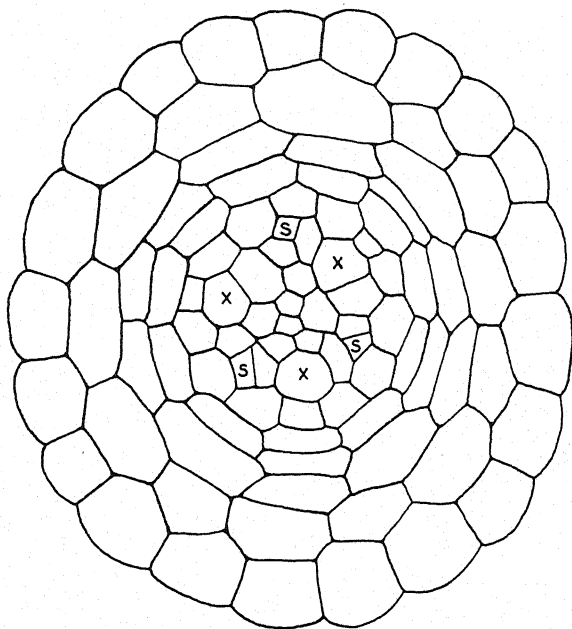


Fig. 6. Cross section of a primary root of *Phleum pratense*, 200 μ from the base of the root cap. The first sieve tubes (S) of the protophloem and the first prospective xylary elements (X) could be recognized at this level. $\times 560$.

cells. This illumination remained constant during the course of each experiment. The lenses employed were a 70 X, 1.25 n. a. Zeiss, water-immersion objective and a 10 X Bausch & Lomb compensating ocular. The laboratory was darkened and the temperature remained between 24° and 25°C.

TABLE 2. *Developmental*

Symbol	Measurement	Dimensions	
x	Distance from root tip	μ	50
r_x	Rate of displacement of x from tip	$\mu/\text{min.}$	0.018
$\frac{dr_x}{dx}$	Rate of elongation	$(\mu/\mu/\text{min.}) \times 10^4$	1.04
l	Average length of epidermal cells with their bases at x	μ	10.0
$\frac{dl}{dx}$	Change in length of epidermal cells	μ/μ	-0.29
$\frac{1}{l} \left[\frac{dr_x}{dx} - \frac{r_x}{l} \cdot \frac{dl}{dx} \right]$	Number new transverse walls in one row of epidermal cells	$(\text{No.}/\mu/\text{min.}) \times 10^5$	1.56
n	Number rows of cells in entire cross-section of root		81
$\frac{n}{l} \left[\frac{dr_x}{dx} - \frac{r_x}{l} \cdot \frac{dl}{dx} \right]$	Number new transverse walls in root	$(\text{No.}/\mu/\text{min.}) \times 10^4$	12.6
D	Diameter of root	μ	127
L	Length of longitudinal walls in cross section ^a	μ	2130
$L \left(\frac{dr_x}{dx} \right)$	Area of longitudinal walls formed	$\mu^2/\mu/\text{min.}$	0.222
$\frac{\pi D^2}{4l} \left[\frac{dr_x}{dx} - \frac{r_x}{l} \cdot \frac{dl}{dx} \right]$	Area of transverse walls formed	$\mu^2/\mu/\text{min.}$	0.198
	Total area of cell walls formed	$\mu^2/\mu/\text{min.}$	0.420
	Per cent of total wall area in transverse walls		47.0

$$^a L = D (1.63\sqrt{n} + 1.91).$$

in length (see Sinnott and Bloch, 1939), are included in the measurements. The curve in this figure represents average cell length at various levels in the root. This curve, with the values for both the ordinate and abscissa corrected for 9.3 per cent shrinkage, is given in figure 5 (see also table 2). It can be seen that the cells are slightly longer at the extreme tip of the root than they are between 100 μ and 200 μ from the apex, and that between 300 μ and 1000 μ from the tip the average length of the cells is progressively and markedly increased.

Employing the method developed on page 37, estimates of the rate of formation of new transverse cell

walls in a single row of epidermal cells have been made. These are given in table 2 and figure 5. Brumfield (1942) has already pointed out that the steady growth rates of these roots indicate an absence of mitotic rhythmicity such as has been reported as occurring in the roots of certain other species (*e.g.*, Kellicott, 1904; Friesner, 1920).

A study of serial cross sections of roots which had been embedded in paraffin showed that there was little increase in the number of longitudinal rows of cells at distances greater than 100 μ from the root apex (see table 2). Hence, we should have a direct gauge of meristematic activity in the epidermal tis-

TABLE 3. *Growth rates of roots grown under various environmental conditions.*

Conditions of growth		Growth rate in mm./hour
In microscopic mounts; roots covered with wet lens paper; illumination as described in text	Average value from figure 3	0.13 ± 0.02
	From Brumfield, 1942, figure 7	0.24
In petri dishes, growing on moist filter paper	In darkness	0.40 ± 0.14
	In red light ^a	0.44 ± 0.17
	In white light ^b	0.41 ± 0.09

^a Light source, a 10-watt Mazda tungsten-filament ruby safe-light at a distance of one foot.

^b Light source, a 25-watt frosted Mazda tungsten-filament lamp at a distance of two feet.

data summarized.

100	150	200	250	300	350	375	400	450	500	600	700	800	900	1000
0.029	0.044	0.070	0.112	0.21	0.40	0.64	1.01	1.40	1.61	1.85	1.97	2.05	2.09	2.11
2.08	4.10	7.28	14.9	22.8	65.1	212.2	108.9	59.4	33.5	18.3	9.6	5.8	3.4	2.4
9.0	8.5	8.6	9.8	12.2	17.0	21.2	25.0	33.0	42.0	62.5	83.0	103.0	123.5	138.0
-0.14	-0.03	0.011	0.038	0.070	0.135	0.154	0.161	0.171	0.189	0.220	0.220	0.196	0.175	0.110
2.81	5.01	7.42	10.77	8.80	19.60	78.00	17.50	-3.97
102	103	104	105	105	105	105	105	105	105	105	105	105	105	105
28.7	51.6	77.2	113.0	92.4	206.0	819.0	184.0
147	159	166	171	174	175	176	176	176	176	176	176	176	176	176
2730	2970	3110	3220	3280	3290	3310	3310	3310	3310	3310	3310	3310	3310	3310
0.568	1.218	2.262	4.80	7.47	21.4	70.2	36.0	19.7	11.1	6.05	3.18	1.92	1.13	0.80
0.475	0.990	1.602	2.46	2.09	4.7	19.0	4.3
1.043	2.208	3.864	7.26	9.56	26.1	89.2	40.3	19.7	11.1	6.05	3.18	1.92	1.13	0.80
45.5	43.5	41.5	33.8	21.9	18.0	21.3	10.7	0

sue, which makes up approximately 37 per cent of the volume of the root. As may be seen in figure 5, the region of most rapid cell division and that of most rapid root elongation coincide.

An examination of longitudinal sections of roots showed that the cells in the underlying tissues were somewhat longer than they were in the epidermis of the same roots (see table 4), and an error will therefore be introduced, if it is assumed that the rate of cell division is the same for these tissues as it is for the epidermis.

From a further study of cross sections, it was possible to determine the level in the root at which the first vascular elements were differentiated (fig. 5). The newest sieve tubes of the protophloem are ma-

tured within about 230 μ of the root apex, distal to the region of most rapid elongation. It is not surprising that protophloem is frequently obliterated by stretching growth (Esau, 1940, 1941, 1943). The youngest xylary elements begin to become vacuolate at about 300 μ and show characteristic thickenings of the secondary walls at a point about 970 μ from the root apex, where little further elongation is taking place. In the maturer portions of the root there is almost no evidence of stretched secondary walls in these xylary elements. Whether these cells should be considered protoxylem or metaxylem is a debatable point (Goodwin, 1942; Esau, 1943).

Following the method developed on page 38, rough estimates have been made of the rate of increase in

TABLE 4. Differences in cell length between the epidermis and the underlying layers of cells, and the percentage of the root composed of each cell layer or region.

Distance from the tip in mm.	Percentage increase in cell length over that of the epidermis				Area of cell layer as per cent of total cross section
	0-1	1-2	2-3	3-35	
Epidermis	37
Outermost layer of cortical cells	11	25	18	14	23
Middle layer of cortical cells	31	37	13	0	14
Innermost layer of cortical cells	49	36	15	23	10
Vascular cylinder	52	63	45	33	16

cell wall area, transverse and parallel to the long axis of the root. The estimates for transverse walls are subject to errors introduced by the assumption that the rate of cell division in all the tissues of the root is essentially the same as that in the epidermis. The values, computed in μ^2 , are given in table 2 and are shown graphically in figure 7. Attention should be called to the progressive decline in the percentage of wall deposited in the transverse plane, from nearly 50 per cent of the total at the root apex, to zero at the point where a cessation of cell division occurs.

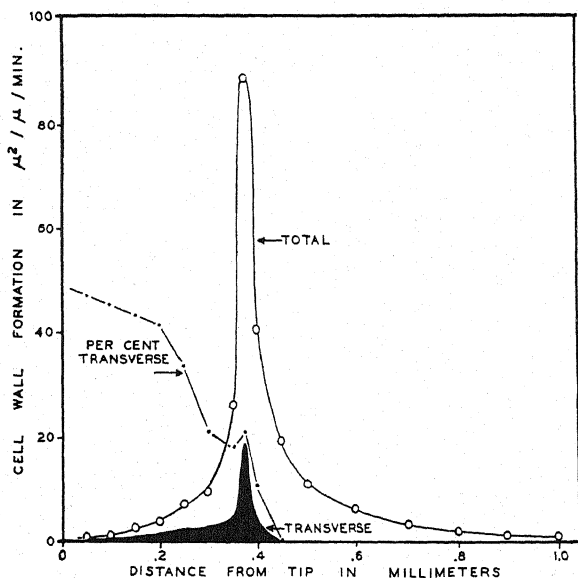


Fig. 7. Estimated rate of increase in total cell wall area (in $\mu^2/\mu/\text{min.}$), plotted as a function of distance from the tip. The estimated rate of increase in area of transverse cell walls (solid black) and the percentage of total wall area laid down as transverse walls are also given.

No accurate measurements have been made of the thickness of the cell walls. If one were to assume some definite thickness for the primary walls shortly after their formation, the data for increase in cell wall area could be used as a rough index of the rate of cellulose deposition at various levels, at least from the root apex back some 700 μ or more, to a point where secondary thickening and the production of root hairs are beginning to take place. It is interesting to note the very low rates of total cell wall formation (and, hence, cellulose deposition), of root elongation and of cell division in the apical 230 μ of the root. The rows of differentiated sieve tubes of the protophloem, which are presumed to be translocating organic food materials to the growing region of the root from the rest of the plant, traverse the most rapidly-growing zone and terminate about 230 μ from the root apex.

DISCUSSION.—Before drawing general conclusions concerning the growth of roots in higher plants, it is obvious that many more studies along the lines of the present investigation should be made on this and

other species. The measurements reported here only apply to primary roots of *Phleum* seedlings of a particular age, grown under one set of environmental conditions. Future studies could, perhaps, best be made by obtaining adequate photographs of the surface of growing roots at short time intervals. Brumfield (1942) has shown that this is technically practical in the case of slender grass roots. The photographic method would have a number of advantages over the method reported here, since measurements of root elongation and of cell length could be made on the same root, and an experimental check on the estimated rates of cell division could also be obtained.

The results of the investigation reported in this paper demonstrate once again the complexity of growth at the meristem. Elongation, cell division, cell enlargement and the differentiation of the various cell types, each of these processes, and a host of others, makes its contribution toward building the intricate structure of the mature root. There is usually a simultaneous operation of several of these processes at any particular level in the root tip, while the rate of each undergoes progressive changes in passing along the root.

In most of our current botanical texts, the root is divided into four regions: the root cap; a meristematic zone, or zone of cell division; a zone of cell elongation, frequently less exactly termed a zone of elongation; and a zone of differentiation or maturation, in which the cells are supposed to become specialized to carry on specific activities. The question may be asked, which of these various regions can be recognized in *Phleum* roots.

The root cap in Phleum is poorly developed, but its boundaries are clearly delineated.

The meristematic zone in this species, if defined as that portion of the root in which transverse cell divisions are taking place, includes the apical 425 μ of root, exclusive of the root cap. This region, in turn, may be roughly subdivided into two parts: (1) the apical 200 μ –300 μ , characterized by slow growth and the presence of "typical" (small, richly protoplasmic, non-vacuolated) meristematic cells; and (2) the basal 125 μ , characterized by extremely rapid elongation and cell division and the presence of vacuolating dividing cells. Priestley (1929), in discussing the nature of the shoot apex in flowering plants, has already emphasized the differences between the apical "meristematic cell" and the sub-apical "vacuolating dividing cell," and it is interesting to note the close correlation between the location of these cell types in the root and the marked changes in growth rate.

Growth processes in the apical portion of the meristem are probably limited by a shortage of food supplies. Even though organic materials may be transported by the matured sieve tubes of the protophloem to within 230 μ of the root apex, from there on, they must pass through a series of undifferentiated cells which are actively utilizing such mate-

rials for their various activities. Hence, a decreasing concentration gradient will be set up, with the most acute shortage at the root apex. Priestley (1928) considers this to be the probable explanation for the relatively slow growth and large size of apical cells in the meristems of many vascular cryptogams. It is not surprising, therefore, that average cell size in the epidermis and in some, if not all, of the underlying tissues is significantly larger at the apex of the root meristem in *Phleum*, and also in other species of grasses (Wagner, 1937), than it is further back along the axis, where more frequent cell division is taking place. These observations are not in complete conformity with the generalization "that minimal cell size occurs in the youngest tissue—the terminus of the meristem . . .—and that there is a steady increase until a rather definite size is reached . . ." (Sinnott, 1938).

In the rapidly-elongating basal portion of the meristematic zone, the rates of cell division and of root elongation appear to be many times as rapid as they are further towards the tip. Here the cells are apparently well supplied with the organic materials necessary for growth by the mature sieve tubes which traverse this region. The fact that the maximum growth rate occurs in the subapical region of the root has been common knowledge, ever since the early marking experiments of Sachs (1882), and has been clearly demonstrated by Brumfield (1942)⁵. The increased rate of cell division in this region, however, was not reported by Brumfield, and would appear to do violence to the concept that "in primary meristems of root and stem it (cell division) is most frequent at the tip and becomes progressively less so with increasing distance back along the axis, until it finally ceases" (Sinnott, 1938). In this basal portion of the meristematic zone there is some increase in average cell size and a beginning of vacuolation of the protoplasts. Here appears to be the principal location of root elongation, which should not be confused with the true zone of cell elongation.

The zone of cell elongation might be considered as falling between about 400 μ and 1000 μ from the base of the root cap. In this zone the root is elongating relatively slowly, and since there is little or no cell division, this elongation is correlated with the presence of "vacuolated extending" cells (Priestley, 1929). Actually, of course, the average length of the cells begins to increase at about 300 μ from the root apex, well within the meristematic zone. It is

⁵ From an analysis of the expansion of cell complexes in the epidermis of *Phleum* roots, Brumfield (1942) came to the conclusion that there were two zones, one between 0 μ and 140 μ and the other between 310 μ and 970 μ of the root apex, each characterized by a constant growth rate (see his fig. 6). This is not in agreement with the data presented in figures 2 and 3 of this paper, which indicate that the growth rate is constantly changing with position in the root. The discrepancy may be due in part to the fact that Brumfield estimated average growth rates from the expansion of cell complexes 100 μ to 600 μ in length, in the older portions of the root, whereas the total length of the most rapidly growing zone may actually be as short as 50 μ .

a mistake to obscure with terminology the fact that there are gradual transitions from one stage of development to another.

The present use of the phrase "zone of elongation" is ambiguous, since it is usually not clear whether the authors using it wish to designate the entire portion of the root which is elongating (in *Phleum*, from about 100 μ to 1000 μ), that portion which is elongating, exclusive of the meristematic zone (in *Phleum*, from about 400 μ to 1000 μ), or that portion which is elongating most rapidly (in *Phleum*, from about 300 μ to 500 μ).

In the authors' opinion the phrases "zone of differentiation" and "zone of maturation" should be avoided, except where a particular cell type is designated. It is clear from the data presented here and elsewhere (e.g., Esau, 1941) that the differentiation of various types of cells occurs at different levels within the root. Sieve tubes, for example, are matured well in advance of xylary elements—in the case of *Phleum*, in the meristematic zone, where organic food supplies should be most rapidly utilized. Xylary elements are differentiated further back, frequently in the zone of cell elongation. The youngest of these elements are probably matured as a functional part of the xylary system near the point where root hairs commence to develop, and where the root becomes prepared to play its absorptive rôle. Root hairs normally develop behind the zone of cell elongation; otherwise, they would be sheared off as the growing portions of the root slid past the soil particles. The portion of the root in which root hairs are formed may aptly be called the *zone of differentiation of root hairs*, and may be readily identified by observation.

SUMMARY

Quantitative measurements of the rate of elongation and of cell enlargement in primary roots of *Phleum pratense* have been made under a standard set of conditions. A method is described for calculating the rate of cell division at various levels in these roots from the above-mentioned data. The relationships between these three developmental processes and the differentiation of the vascular elements at various levels within the root have been graphically represented in figure 5. Estimates of the rate of increase in area of the transverse and longitudinal cell walls formed at various levels have also been made and are summarized in figure 7.

Under the particular experimental conditions described, four regions may be distinguished in the growing root tip. They are: (1) the root cap; (2) the apical 300 μ of the meristematic zone, characterized by slow elongation, slow cell division and the presence of typical meristematic cells; (3) the adjacent 125 μ at the base of the meristematic zone, characterized by rapid elongation, rapid cell division and the presence of vacuolating dividing cells; and (4) the so-called zone of cell elongation, falling between about 400 μ and 1000 μ from the base of the

root cap, and characterized by relatively slow elongation, absence of cell division and, hence, the presence of vacuolated, extending cells.

Cell differentiation occurs at different levels within the root, depending upon the particular type of cell involved. Thus, sieve tubes are matured within 230 μ of the base of the root cap, well within the meristematic zone; while the youngest xylary elements show characteristic thickenings of the sec-

ondary walls are found at a point 740 μ further back, in the zone of cell elongation. For this reason, the use of the terms "zone of differentiation" and "zone of maturation," without designating a specific cell type, should be discontinued.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF ROCHESTER,
ROCHESTER, NEW YORK

LITERATURE CITED

- BRUMFIELD, R. T. 1942. Cell growth and division in living root meristems. *Amer. Jour. Bot.* 29: 533-543.
- ESAU, K. 1940. Developmental anatomy of the fleshy storage organ of *Daucus carota*. *Hilgardia* 13: 175-209.
- . 1941. Phloem anatomy of tobacco affected with curly top and mosaic. *Hilgardia* 13: 437-470.
- . 1943. Origin and development of primary vascular tissues in seed plants. *Bot. Rev.* 9: 125-206.
- FRIESNER, R. C. 1920. Daily rhythms of elongation and cell division in certain roots. *Amer. Jour. Bot.* 7: 380-407.
- GOODWIN, R. H. 1942. On the development of xylary elements in the first internode of *Avena* in dark and light. *Amer. Jour. Bot.* 29: 818-828.
- KELLCOTT, W. E. 1904. The daily periodicity of cell division and of elongation in the root of *Allium*. *Bull. Torrey Bot. Club* 31: 529-550.
- LEWIS, F. T. 1943. The geometry of growth and cell division in epithelial mosaics. *Amer. Jour. Bot.* 30: 766-776.
- PRIESTLEY, J. H. 1928. The meristematic tissues of the plant. *Biol. Rev.* 3: 1-20.
- . 1929. Cell growth and cell division in the shoot of the flowering plant. *New Phytol.* 28: 54-81.
- SACHS, J. 1882. *Vorlesungen über Pflanzen-Physiologie*. Leipzig.
- SINNOTT, E. W. 1938. Structural problems at the meristem. *Bot. Gaz.* 99: 803-813.
- . 1939. Growth and differentiation in living plant meristems. *Proc. National Acad. Sci. U.S.A.* 25: 55-58.
- , AND R. BLOCH. 1939. Cell polarity and the differentiation of root hairs. *Proc. National Acad. Sci. U.S.A.* 25: 248-252.
- , AND ———. 1941. The relative position of cell walls in developing plant tissues. *Amer. Jour. Bot.* 28: 607-617.
- WAGNER, N. 1930. Über die Mitosenverteilung im Meristem der Wurzelspitzen. *Planta* 10: 1-27.
- . 1937. Wachstum und Teilung der Meristemzellen in Wurzelspitzen. *Planta* 27: 550-582.

CONTROLLING THE pH OF CULTURES OF *PENICILLIUM NOTATUM* THROUGH ITS CARBON AND NITROGEN NUTRITION¹

Albert E. Dimond and George L. Peltier

A SURVEY of published material reveals relatively little information concerning the nutritional physiology of *Penicillium notatum* Westl. on synthetic media. Metabolism studies of *P. notatum* on various media have appeared to be incidental to the more pressing problem of producing penicillin quickly and in quantity. Fleming (1929) originally used a nutrient broth non-synthetic in nature. The Oxford group (Abraham *et al.*, 1941) then adopted a modification of the so-called Czapek-Dox synthetic medium which supplies nitrogen as sodium nitrate and glucose as carbohydrate. Hobby, Meyer, and Chaffee (1942) suggested that brown sugar be substituted for glucose in this nutrient, with resultant higher yields and decreased time for maximum production of penicillin. Apparently a variety of carbon and nitrogen sources have been employed in liquid nutrients (Foster, 1943; Foster, Woodruff and McDaniel, 1943; McKee and Rake, 1942; Kocholaty, 1942; Waksman and Horning, 1943); but specific data have not been offered. At the present time corn steep liquor appears to be an important component of the medium used.

It is the purpose of the present paper to discuss the relation between the period of incubation and the pH trend in nutrient solutions, as influenced by the composition of the nutrient on which *P. notatum* is grown. As these studies will show, the pH developed in liquid culture can be well regulated by supplying the organism with appropriate sources of carbon and nitrogen. Such a possibility would seem of value in the production of penicillin, in view of the fact that the pH of culture media must be kept in the range of 5.5 to 7.5 for maximum production (Abraham *et al.*, 1941).

EXPERIMENTAL.—In this study a "surface" strain of *P. notatum* was grown in wide-mouth gallon jars having an internal diameter of 15 cm. The maximum depth of the medium was 10 cm. so that the surface: volume ratio was 0.1. Two liter quantities of media were added to each jar, sterilized, and inoculated with spore suspensions from several cultures of *P. notatum*. The incubation temperature varied from 25° to 28°C. Two days after inoculation, the surface mycelium was usually sufficiently heavy so that the culture medium could be gently and continuously stirred below by means of an aseptic stirring device

¹ Received for publication June 28, 1944.

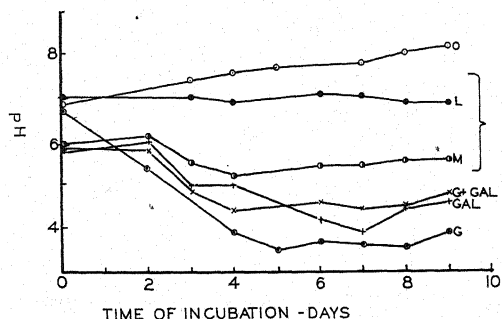
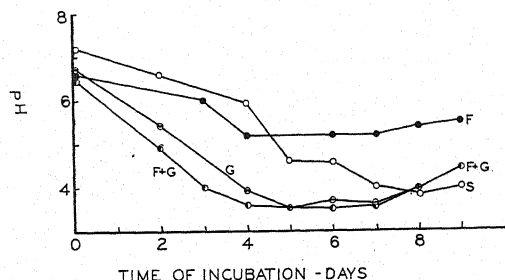
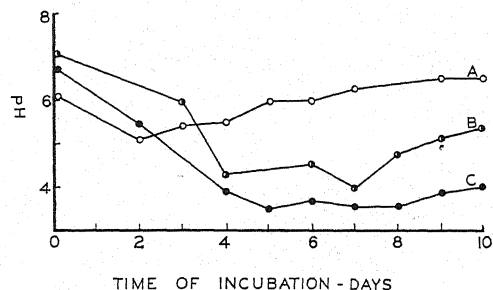
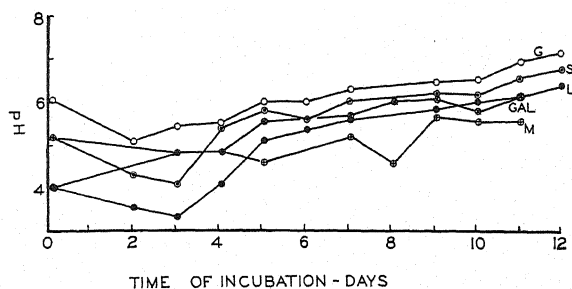


Fig. 1-4.—Fig. 1 (uppermost). Relation of time of incubation to pH developed in liquid culture by *P. notatum* when NaNO_3 is the source of nitrogen and different sugars are supplied. Curve G is curve for glucose, S for sucrose, L for lactose, GAL for galactose and M for maltose.—Fig. 2. Relation of time of incubation to pH developed in liquid culture by *P. notatum* when glucose is the sugar supplied and varying sources of nitrogen are used. Curve A is obtained when NaNO_3 is supplied, B when a mixture of NaNO_3 and the amino acids tryptophane, asparagine, and cystine are available, and C when these amino acids only are present.—Fig. 3. Relation of time

without hindering further growth of the fungus pad. Except for media containing lactose, subsequent growth was on the surface of the medium. Stirring was effective in hastening penicillin production, so that the peak was reached in eight to nine days on all media in which penicillin was produced. Through the cotton plug in the mouth of the culture jar were placed Y tubes of such construction that 10 ml. samples of culture medium would be withdrawn for use in measuring pH of the culture medium without contaminating the culture (fig. 3 in Beckord, 1943).

All chemicals employed were of reagent grade. Two types of media were employed for the work, the first being a slight modification of the medium commonly used (Hobby, Meyer, and Chaffee, 1942) in experiments reported in the literature, consisting of 3.5 g. NaNO_3 , 1.5 g. KH_2PO_4 , 0.5 g. KCl, 0.5 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 20 g. of pure sugar and trace elements (Cu, Zn, Mo, Mn, Fe, B). These salts were diluted with a liter of distilled water. The second medium (Burkholder and McVeigh, 1942) was similar except that NaNO_3 was omitted and in its place 2.6 g. asparagine, 0.1 g. tryptophane, and 0.05 g. cystine were substituted.

In some instances media were prepared in duplicate within a single experiment; in others single batches of media were employed for each experiment. All results reported are based on at least three independent tests. The results from test to test were always consistent.

Results.—Nitrogen sources and change in pH.—The pH trend with time in all cultures which contained NaNO_3 as a source of nitrogen was upward from an initially acid reaction (see fig. 1). Under these conditions the pH slowly rose from a value as low as 4.0 toward the range favorable for penicillin production, arriving in this neighborhood at least by the eighth day. Thus, when nitrate was the source of nitrogen, the trend in pH was apparently determined by use of nitrate ion at a more rapid rate than sodium ion, while the effect of sugar metabolism on pH was secondary (fig. 1 and 2).

With the amino acids, the trend in pH with growth of the fungus was in the opposite direction, i.e., downward, when glucose and sucrose were the sugars supplied (fig. 3, curves G and S). The down-

ward trend in pH developed in liquid culture by *P. notatum* when the source of nitrogen is a mixture of the amino acids tryptophane, asparagine, and cystine, and when different sugars are employed. Curve F is for fructose, curve F + G is for a mixture of equal quantities of fructose and glucose, curve G is for glucose, and curve S is for sucrose.—Fig. 4 (lowermost). Relation of time of incubation to pH developed in liquid culture by *P. notatum* when the source of nitrogen is a mixture of the amino acids tryptophane, asparagine and cystine, and when the sugar supplied was varied. Curve O was obtained when no sugar was supplied. Curve L is for lactose, M for maltose, G + GAL for a mixture of equal quantities of glucose and galactose, GAL for galactose alone, and G for glucose alone. The region enclosed within the brackets is the region most favorable to production of penicillin.

ward trend of pH with growth was more pronounced for the glucose medium than for sucrose.

Figure 2 contrasts nitrates and amino acids as nitrogen sources in liquid media, with glucose as the sugar source. Similar results were obtained when sucrose was used. In figure 2, curve A was obtained with nitrate as the sole N source, while curve C was obtained when amino acids were supplied. Inasmuch as the pH trends are opposite in direction in these two instances, it seemed of interest to test whether or not an intermediate type of curve might be obtained when amino acids and nitrate ion were supplied together in the medium. Curve B of figure 2 is the typical curve noted for this situation, nitrate being present at the rate of 2 gms. per liter. In such a case the drop in pH is initially almost as rapid as for a medium supplying nitrate only. However, the pH does not finally drop so low, and starts to rise sooner than the curve for nitrates. Under these conditions the pH apparently remains low until most of the amino acids present are utilized.

Carbon sources and change in pH.—Various carbohydrates were used as constituents of the nitrate medium for *P. notatum*. In all cases, the trend was that already noted for glucose and sucrose. In figure 1 are given the trends of pH with time when the sugars making up the medium were respectively glucose, sucrose, lactose, galactose, and maltose. Similar changes were observed for fructose and brown sugar.

Figures 3 and 4 indicate the results of typical experiments for all sugars tested on the amino acid medium except brown sugar. Brown sugar and sucrose showed the same trend in pH, though growth was somewhat more rapid on brown sugar.

The contrast in the pH curves for various sugars is striking. Variation ranged from practically no change in pH on incubation (lactose) to a rapid drop in pH and gradual recovery to pH 4 (glucose). Nutrients containing maltose and fructose became slightly more acid as growth of *P. notatum* went on, while solutions containing galactose and sucrose as well as those containing equal parts of fructose and glucose, glucose and galactose became acid more rapidly.

In figure 4 are plotted the curves for lactose, maltose, glucose, galactose, and a mixture of glucose and galactose. In the mixture each sugar is added at half the normal concentration. Considering the family of curves consisting of lactose, galactose, glucose and the mixture of galactose and glucose, one can see that these curves form three separate groups. These groups are (1) lactose alone, (2) glucose alone, and (3) galactose alone and glucose plus galactose. No change in pH occurred during the normal course of metabolism on lactose. Curve G for glucose contrasts with this, for the pH changed from the neighborhood of neutrality to pH 3.5 in about five days. The curves for galactose and for glucose plus galactose are similar to one another. For these the pH tends to drop more slowly than for

glucose alone and drops only to pH 4.5 in the same period.

DISCUSSION.—Nitrogen sources and change in pH.

—The striking point about the curves relating pH of the culture medium to time of incubation for different sources of nitrogen (fig. 1, 2, and 3) is that the pH of the medium can be controlled by supplying the appropriate nutrients. By supplying graded quantities of nitrate and amino acids, any desired pH for a particular stage of the growth period can be obtained when a sugar is used which is metabolized to organic acids. Reasons for such behavior are not hard to find. Foster, Woodruff, and McDaniel (1943) have ascribed the initial drop in pH in nitrate media to formation of gluconic acid from glucose or sucrose, and the gradual trend back toward neutrality to the absorption and metabolism of nitrate ion without a correspondingly rapid utilization of the sodium ion. The latter presumably becomes associated with hydroxyl ion from water in the nutrient and pH rises as these accumulate. This trend of pH with time is characteristic of nitrate metabolism in plants (Nightingale, 1937). The trend toward decreasing pH values with amino acids might be similarly considered as due to the accumulation of organic acids resulting from the deamination of amino acids and from formation of gluconic acid when glucose is the source of carbon. Thus, one might expect a more rapid and greater drop in pH when amino acids are the nitrogen source than when nitrates are supplied.

The trend for the intermediate curve might well depend on which form of nitrogen was most readily used by the fungus. In general, amino acids appear to be used selectively when both NO_3 ion and amino acids are available. The organic acids from amino acids may be used as the carbon source more rapidly than glucose, after the glucose concentration has been reduced somewhat by metabolism with the result that pH remains stable. All the curves in figure 4 indicate that the pH does remain stable from the fifth day onward. Much more rapid growth occurred on the amino acid medium than on the nitrate medium.

Carbon sources and change in pH.—A comparison of the various curves in figures 3 and 4 indicates something as to the metabolism of the sugars involved. In figure 3 are compared the pH trends for sucrose, fructose, glucose, and a mixture of equal parts of glucose and fructose. The last nutrient contained each sugar at half the usual concentration, and would be the mixture obtained if sucrose were hydrolyzed initially to its constituent monosaccharides. The pH trend for sucrose is somewhat different from that for the mixture of the two monosaccharides. The curve for sucrose is distinct from that for fructose. Each differs from that of glucose alone or of glucose and fructose in equal quantity. In the mixture of glucose and fructose, glucose appears to be the principal sugar metabolized, whereas the fructose is used slightly if at all. This is indi-

cated by the similarity of curve F + G and curve G and the contrast between these and curve F as shown in figure 3. Other aspects of the behavior of *P. notatum* growing on these nutrients give evidence for the relative unavailability of fructose. The growth of *P. notatum* on nutrients containing fructose alone is slow and the nutrient becomes gradually colored by a black pigment. No such pigmentation occurs when the nutrient contains a mixture of fructose and glucose and growth is almost as rapid as in a nutrient containing glucose alone.

It appears as though sucrose is gradually hydrolyzed to fructose and glucose, and the glucose then proceeds to the gluconic acid stage as a subsequent step. This picture of sucrose metabolism would account for the gradual drop in pH with time as contrasted with the more rapid drop in media containing glucose and fructose together or glucose only.

From figure 4 it can be inferred that glucose is used rapidly in growth, presumably with formation of gluconic acid. Likewise acid is formed as galactose is used. When a mixture of glucose and galactose is available, the galactose appears to be used first, since the time *vs.* pH curve of the mixture is almost that for galactose alone. The curve for lactose is of interest since there is practically no change in pH with time in this medium. On the lactose-amino acid medium, growth is slower in starting than for other sugars, but penicillin activity, as measured from the crude filtrates in these tests, appeared both earlier and in greater quantity than with any other sugar tested. Figure 4 indicates that pH remains fixed within the optimum range for penicillin formation in this medium. There are several possible interpretations of the difference between the curve for lactose utilization and for curves of the related sugars. Lactose may be utilized very slowly and completely as it is broken down to the monosaccharides without accumulation of sugar acids. A second possibility is that lactose is not hydrolyzed to constituent sugars, or if hydrolyzed, the corresponding sugar acids are not formed in the resulting metabolism. The third possibility is that lactose is not used at all by the fungus, but that the fungus obtains its carbon from the amino acids.

The medium consisting of the mixture of glucose and galactose is approximately the medium which would result from hydrolysis of lactose. If the course of lactose utilization involves hydrolysis, then one might logically expect a gradual drop in pH with time, since a nutrient composed either of glucose, galactose, or a mixture of these two becomes more acid with time. Though the drop in pH with time in a lactose medium might be slower than in a mixture of glucose and galactose owing to gradual breakdown of lactose, there would still be a drop in pH with time. Figure 4 shows that no such accumulation of hydrogen ions occurs and this leads to the conclusion that lactose is not hydrolyzed to constituent sugars in its metabolism.

Slow growth by the fungus on an amino acid-lactose medium suggests that lactose may not be utilized. To test this point, growth of the fungus was observed on a medium containing amino acids without sugar. The resulting curve of pH with time is curve "O" in figure 4. This curve indicates a gradual but definite rise in pH with time, starting with a neutral reaction and ending in excess of pH 8. Evidently, then, lactose is slowly used by the fungus. In the medium lacking sugar, the gradual rise of pH with time is interpreted as being the result of more rapid utilization of the carbon chain of the amino acids than the amino nitrogen of the molecule. Thus, there is a gradual accumulation of basic nitrogen in the nutrient solution, resulting in a rise in pH.

The pair of curves (fig. 4) describing the change of pH with time for nutrients containing maltose on the one hand and glucose on the other presents a similar problem. There is marked contrast between these two curves and little if any accumulation of acid in the medium supplying maltose as a sugar. Since growth is rapid on the medium, maltose must be used by the fungus. Moreover, there is a difference between the pH *vs.* time curve in media containing amino acids with and without maltose (curves M and O). It would seem, again, that, if maltose were hydrolyzed to glucose in metabolism, gluconic acid would accumulate and there would be a more rapid drop in pH than was observed in this medium.

The data presented indicate that pH of the nutrient medium can be regulated without addition of acids or alkalies by merely supplying the fungus with the proper sources of carbon or nitrogen. Addition of acids and alkalies is of questionable value in production of penicillin, for in our tests, no penicillin was produced in media in which pH was controlled by means of this technique.

Growth in the presence of mass inocula is very rapid on an amino-acid sugar medium. The use of yeast extract has been suggested to hasten the production of penicillin (Clifton, 1943). It may be that the organic nitrogen introduced in this process is sufficient to cause an initially rapid growth, which is followed by more leisurely absorption of nitrate, once the fungus pad is established. In this latter phase of growth, pH of the culture medium would be rendered favorable to penicillin production. This hypothesis is strengthened by the work of Kocholaty (1942) which indicates that the usual growth factors are not important in the metabolism of *P. notatum*.

SUMMARY

The present investigation concerned itself with the relation between pH of the nutrient medium and time of incubation, particularly as this relation is affected by varying the source of nitrogen or sugar.

When nitrate nitrogen was supplied, the pH first dropped slightly, then gradually rose to the range

favorable for penicillin production. This was observed for sucrose, lactose, glucose, brown sugar, maltose and galactose.

When amino acids were present, the relation of pH to time of incubation varied with the sugar. For lactose, the pH remained practically constant at pH 7.0. With maltose, the pH dropped slightly to about 6.0 and remained constant. For fructose the same type of curve was obtained. For sucrose, glucose, galactose and mixtures of glucose and fructose as well as glucose and galactose, the pH fell to the range of 3.5 to 4.5 and remained there until autolysis began. The relation of this behavior to sugar metabolism is discussed.

When amino acids and nitrate nitrogen were supplied together, the pH fell somewhat, but neither

to so great an extent as with nitrate alone or amino acids alone and rose again to a level intermediate between that observed for nitrate alone and amino acids alone.

These results suggest that by supplying the proper nutrients to cultures of *P. notatum*, control of the pH of the culture medium may be maintained. The relation of pH to the production of penicillin is by now well known. Such techniques for control of pH, together with the fact that growth becomes rapidly established on amino acid media may find use in production of penicillin.

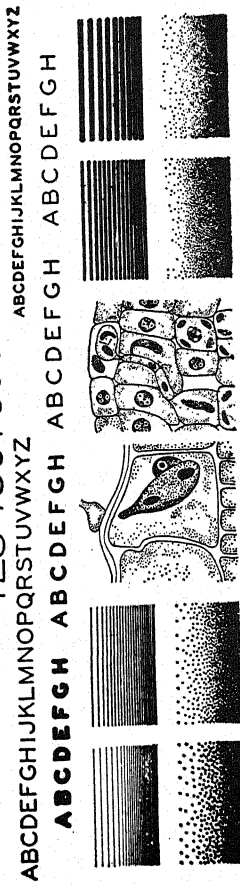
DEPARTMENTS OF BOTANY AND BACTERIOLOGY,
UNIVERSITY OF NEBRASKA,
LINCOLN, NEBRASKA

LITERATURE CITED

- ABRAHAM, E. P., E. CHAIN, C. M. FLETCHER, A. D. GARDNER, N. G. HEATLEY, M. A. JENNINGS, AND H. W. FLOREY. 1941. Further observations on penicillin. *Lancet* 241:177-182.
- BECKORD, L. D. The production of bacterial amylases. Fig. 3, p. 17. M. A. Thesis. University of Nebraska. July, 1943.
- BURKHOLDER, P. R., AND I. McVEIGH. 1942. Synthesis of vitamins by intestinal bacteria. *Proc. National Acad. Sci. U.S.A.* 28:285-289.
- CLIFTON, C. E. 1943. Penicillin production on a large scale. *Science* 98:69-70.
- FLEMING, A. 1929. On the antibacterial action of cultures of a *Penicillium*, with special reference to their use in isolation of *B. influenzae*. *Brit. Jour. Exp. Path.* 10:226-236.
- FOSTER, JACKSON W. 1943. Microbiological aspects of penicillin formation (abstract). *Jour. Bact.* 45:65.
- , H. BOYD WOODRUFF, AND L. E. McDANIEL. 1943. Microbiological aspects of penicillin. III. Production of penicillin in subsurface cultures of *Penicillium notatum*. *Jour. Bact.* 46:421-433.
- HOBBY, G. L., K. MEYER, AND E. CHAFFEE. 1942. Activity of penicillin *in vitro*. *Proc. Soc. Exp. Biol. and Med.* 50:285-288.
- KOCHOLATY, WALTER. 1942. Cultural characteristics of *Penicillium notatum* in relation to the production of antibacterial substance. Indication of the dual nature of the antibacterial substance. *Jour. Bact.* 44:469-477.
- McKEE, C. M., AND GEOFFREY RAKE. 1942. Biological experiments with penicillin (abstract). *Jour. Bact.* 43:645.
- NIGHTINGALE, GORDON T. 1937. The nitrogen nutrition of green plants. *Bot. Rev.* 3:85-174.
- WAKSMAN, S. A., AND E. S. HORNING. 1943. Distribution of antagonistic fungi in nature and their antibiotic action. *Mycologia* 35:47-65.



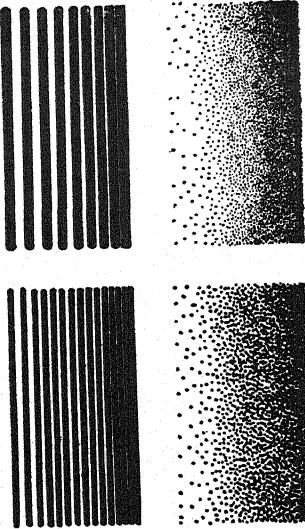
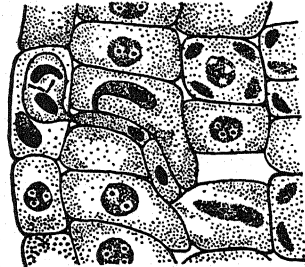
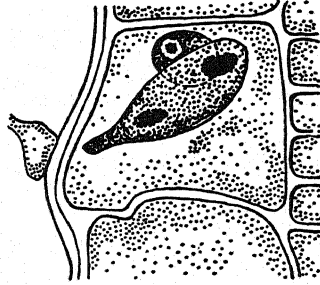
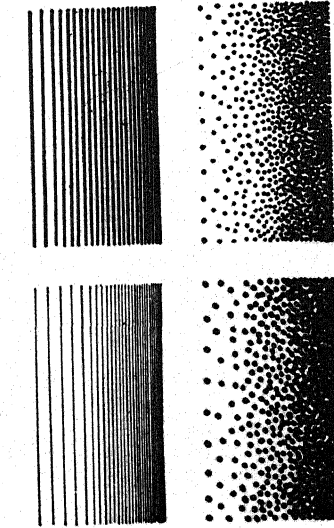
ABCDEFGHIJKLMNOPQRSTUVWXYZ
 1234567890



ABCDEFGHIJKLMNOPQRSTUVWXYZ
 1234567890

ABCDEFGHIJKLMNOPQRSTUVWXYZ

ABCDEFGH ABCDEFGH ABCDEFGH



ABCDEFGHIJKLMNOPQRSTUVWXYZ

ABCDEFGH ABCDEFGH

DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

Reprinted by permission of the authors from: RIKER, A. J., and REGINA S. RIKER. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

THE BREEDING OF ORNAMENTAL EDIBLE PEACHES FOR MILD CLIMATES.

I. INHERITANCE OF TREE AND FLOWER CHARACTERS ¹

Walter E. Lammerts

THE RAPIDLY increasing population of Southern California has resulted in a great increase in suburban homes with relatively small planting space. Accordingly, dual purpose trees combining ornamental value with the production of edible fruit are much in demand. Double flowering peach trees are the most popular of the ornamental deciduous trees because of their rapid growth, great profusion of bloom and ease of culture. Unfortunately, most varieties bear small, fuzzy, clingstone fruits with unattractive skin color and rather bitter flavor. The one exception seems to be the variety Aurora which has a medium size white freestone fruit, which, though lacking in color, is comparatively free of bitterness. Occasionally other trees are reported but all so far found are small, unattractive in color, and at best only fair in flavor. In order to develop ornamental varieties with larger freestone fruits, having good skin color and flavor, hybridization of the best commercial peaches with double flowering varieties was begun in the spring of 1936. In this report, only the data on the inheritance of tree and flower characteristics found to be of importance in accomplishing this objective are presented.

MATERIAL AND METHODS.—The Babcock peach was selected as the white-fleshed commercial variety, because it has a well-shaped fruit of excellent skin color and sweet flavor. It was crossed with the Early Double Pink and Early Double Red varieties in the spring of 1936. These varieties have a short chilling requirement and so flower very uniformly and abundantly after even our mildest winters. Their clingstone fruits are very bitter, small and fuzzy, with the skin completely lacking in color. The Babcock was used as the female parent in these crosses because of the relatively high percentage of flowers which set fruit. The Rio Oso Gem was selected as one of the yellow fleshed commercial varieties because it has very large, high quality fruit when grown in regions with sufficient winter chilling. The July Elberta was used as the other yellow fleshed variety because it has a medium large fruit of good quality, even in Southern California, mainly due to its chilling requirement being short enough to be satisfied by our mild winters. Both Rio Oso Gem and July Elberta were crossed to the ornamental

Chinese Dwarf Mandarin which has rather small, double red flowers, is practically evergreen especially near the coast, and has a very short chilling requirement. By intercrossing F_2 selections of these hybrids with those obtained from Babcock x Early Double Pink and Red, both semi-evergreen and deciduous, yellow and white fleshed, double flowering varieties of good quality have been obtained. Some of these F_2 seedlings have recently been crossed to very early flowering seedlings of St. Helena, another evergreen variety having small freestone yellow fruit, and to derivatives of Babcock x Mayflower having a very short maturity cycle, in hopes of developing early fruiting ornamental varieties.

The fruit of the above crosses was harvested at maturity and, as already reported (Lammerts, 1942), most of the seeds were embryo-cultured. Very high germination percentages (often 100 per cent) were obtained, and the trees from crosses made in 1936 were indexed as to leafing and flowering characteristics in the spring of 1938. Since a short chilling requirement and good flower character were two of the main objectives of the breeding work, full advantage could be taken of the short breeding cycle resulting from the use of the embryo culture technique. Selections were regularly made two years after crossing in the spring at flowering time and these selections were immediately used in selfing and backcrossing. Thus, selected trees from the 1936 crosses were selfed and backcrossed in the spring of 1938. In the summer of 1938 further selections, based on fruit quality, were made and these were selfed and backcrossed in the spring of 1939. The F_2 and backcross progenies resulting from the 1938 pollinations were indexed in the spring of 1940 and those from the 1939 pollinations in the spring of 1941. Except where noted, all populations reported in this paper were grown at the Armstrong Nurseries, Ontario, California.

THE F_1 HYBRIDS.—None of the 33 F_1 hybrids of Babcock x Early Double Pink² leafed and flowered as early as Early Double Pink, which began leafing and flowering January 22, 1938. The spring of 1938 followed a very mild winter during which only 406 40° F. hours occurred (Lammerts, 1941). As shown in table 1, only one hybrid leafed out and flowered by February 10. Another group of ten leafed out and flowered during the period from February 22–27, and four hybrids did not begin leafing or flowering until March 15–25, later than Babcock which began flowering March 10, though

² Except where otherwise noted, the variety mentioned first was used as the female parent.

¹ Received for publication August 19, 1944.
The author is indebted to the Armstrong Nurseries, Ontario, California, for facilities used in growing and maintenance of hybrid progenies while employed by them from 1935–1940, and especially to Herbert Swim of the Armstrong Nurseries for his cooperation in making it possible for the author to continue his observations of these progenies while employed by the University of California, Los Angeles, since 1940.

TABLE 1. Leafing out dates of F_1 hybrids obtained by crossing indicated low chilling double flowering varieties with high chilling commercial varieties. The number of 40° F. hours in years when records were taken are also given. Records taken at Ontario, California.

Low chilling double varieties	Leafing out date	F_1 hybrids grouped according to indicated leafing out dates	Total	High chilling commercial varieties	Leafing out date	No. hours at or below 40° F. Nov. 1–Mar. 1
Early Double Pink ♂	1/22/38	2/10/38(1), 2/22–27(10), 3/5–9(18), 3/15–25(4)	33	Babcock ♀	3/26/38	406
Early Double Red ♂	2/24/38	2/21–26/38(3), 3/4–9(15), 3/10–20(11), 3/25–30(17)	46	Babcock ♀	3/26/38	406
Chinese Dwarf Evergreen ♀	1/24/39 ^a	1/22–29/39(4), 2/9(1), 2/27(6)	11	Babcock ♂	3/24/39	647
Chinese Dwarf Evergreen ♀	1/24/39	2/4–9/39(2), 2/25–27(4)	6	Socala ♂	3/25/39	647
Chinese Dwarf Evergreen ♀	1/24/39	2/27–28/39(13)	13	Rio Oso Gem ♂	4/15/39	647

^a Date refers to leafing of lateral buds; terminals remain evergreen except in coldest years at Ontario, California.

delayed in leafing until March 26. The results are substantially the same in the crosses of Chinese Dwarf Evergreen with Socala and Rio Oso Gem. Evidently, then, the short chilling requirement of varieties such as Early Double Pink is not inherited as a dominant factor. In Southern California, time of flowering is dependent on the satisfaction of the chilling requirement, although usually occurring earlier than leafing, since flower buds require less chilling than leaf buds. Though unusually warm spring weather causes earlier flowering, the response is inversely proportional to the chilling requirements. Therefore, edible, very early, double flowering varieties can only be obtained by growing rather large selfed progenies.

All of the above hybrids as well as those obtained by crossing Chinese Dwarf Mandarin with Rio Oso Gem, had flowers much larger than Babcock and slightly larger than Rio Oso Gem. The flower color was darker pink than is characteristic of Babcock. The F_1 population of Chinese Dwarf Mandarin x July Elberta segregated into the large or "showy" flower class and the small or "non-showy." All F_1 hybrids had single flowers with the exception of a few trees in the Babcock x Early Double Pink and Red populations. These trees had occasional flowers with 1–3 extra petals or petaloids. However, as may be seen by referring to tables 3 and 4, these trees did not differ in their transmission of the double flowering character from those showing only single flowers, so the occurrence of these occasional flowers with extra petals probably does not signify a different genetic constitution.

The F_1 hybrids of Chinese Dwarf Mandarin x Rio Oso Gem, Babcock and July Elberta, were completely deciduous and normal in height.

THE F_2 AND BACKCROSS PROGENIES.—(1) *Tree characteristics*.—(a) *Dwarf vs. normal habit*. An F_2 population of Chinese Dwarf x Rio Oso Gem consisting of 71 plants, showed a very clear-cut 3:1 segregation into two classes, i.e., 55 plants were

normal in height and 16 were very small brachytic dwarfs, having very short internodes like the Chinese Dwarf Evergreen parent. The normal type, at the end of two years from culturing of the seeds, varied in height from five to seven feet while the dwarfs were only one to one and one-half feet high. As there were about an equal number of deciduous and evergreen dwarfs, no linkage between the evergreen character and dwarf habit is indicated. This dwarf type is then inherited as a simple recessive and may be denoted by the symbol *dwdw*, the tall normal type of peach being *DwDw*. The *dw* factor causes a more extreme type of dwarfing than is characteristic of the Japan Dwarf Blood used by Blake (1933).

When the Babcock peach was close pollinated, a population of 329 seedlings segregated into 308 tall normal to 21 somewhat brachytic, bushy types with shorter internodes and thickened branches. These grew to a height of only two and one-half to three feet at the end of the two-year growth period. The theoretical expectation, on the basis of a dihybrid 15:1 ratio, is 308.5 to 20.5. Evidently, then, this bushy, compact-growing type is the result of the interaction of duplicate, recessive factors neither one of which is able to cause any readily appreciable decrease in height when acting alone. The F_1 hybrids of Chinese Dwarf Evergreen x Babcock were normal in height, indicating that neither of these duplicate factors is allelomorphic to the *dw* factor. The bushy type is then of the genetic constitution *bu₁bu₁ bu₂bu₂*, and the normal type is *Bu₁Bu₁ Bu₂Bu₂*. The factorial situation relative to tree height as far as analyzed is then as follows:

Tall normal	<i>DwDw Bu₁Bu₁Bu₂Bu₂</i>
Bushy compact	<i>DwDw bu₁bu₁bu₂bu₂</i>
Dwarf	<i>dwdw Bu₁Bu₁Bu₂Bu₂</i>

Since Babcock is heterozygous for the duplicate bushy factors, about one-fourth of the F_1 hybrids obtained by crossing to Early Double Red and Pink (tall normal) would be expected to segregate for

TABLE 2. Leafing out dates of F_2 hybrids obtained by selfing indicated F_1 hybrids. Leafing out dates of seedlings from *St. Helena*, *Babcock*, and *Rio Oso Gem* close pollinated are also given. Records were taken at Ontario, California, except in the case of *St. Helena* seedlings grown at West Los Angeles Station.

	Progeny No. of F ₁ hybrids selfed	Leafing out date	F ₂ hybrids grouped according to indicated leafing out dates	Total	No. hours at or below 40°F. Nov. 1–Mar. 1
Babcock ♀ x Early Double Pink ♂ F ₁ hybrids selfed	36058/2	2/10/38	1/30/42(35), 2/10–11(2), 2/15–22(3)	40	942—1942
	36058/2	2/10/38	1/25/41(1), 2/1(1), 2/3(4), 2/5(1)	7	363—1941
	36058/11	3/6/38	1/30/42(5), 2/2–3(4), 2/5–8(19), 2/11–15(10), 2/26–28(4), 3/6(3)	45	942
	36058/16	2/22/38	2/3/41(2), 2/6(1), 2/12–15(4), 2/20(1)	8	363
	36058/28	2/27/38	1/27–30/42(15), 2/5–8(5), 2/11–15(4), 2/19–22(2)	26	942
	36058/33	2/24/38	1/30/42(6), 2/7–10(6), 2/13(2), 2/22(1)	15	942
	36057/4	2/21/38	2/6/41(1), 2/10–12(5), 2/18–20(9), 2/22–24(5), 3/10(1)	21	363
Babcock ♀ x Early Double Red ♂ F ₁ hybrids selfed	36057/4	2/21/38	1/25–2/1/42(23), 2/4–8(19), 2/10–12(7), 2/23–28(9)	58	942
	36057/14	3/2/38	2/3–4/41(3), 2/6(1), 2/13–15(5), 2/23–25(2), 3/8(1)	12	363
	36057/17	3/7/38	1/30/42(10), 2/3(5), 2/7(2), 2/11–15(13), 2/26–27(6)	36	942
	36057/22	3/4/38	2/1/41(1), 2/9(1), 2/18–20(4), 2/24–26(2), 3/8(1)	9	363
	36057/23	3/2/38	2/6/41(1), 2/10–15(4), 2/21–24(8), 3/10–12(3)	16	363
Chinese Dwarf ♀ x Rio Oso Gem ♂ 37141/8	2/29/40	1/16/43(5), 1/23–25(4), 1/31(5), 2/4–10(3), 2/12–16(24), 2/18–20(22), 2/27–3/1(2), 3/9–10(2), 3/17–19(3)	70 ^a	584—1943	
Babcock close pollinated	3/26/38	2/20–3/4/38(16), 3/15–20(180), 3/21–26(57), 3/27–4/1(61), 4/1–5(38), 4/6–10(2)	354	406—1938	
Rio Oso Gem close pollinated	4/15/39	3/22–24/39(9), 3/29–31(15), 4/3–5(11)	35	647—1939	
St. Helena close pollinated	1/10/41	1/7–9/44(10), 1/13–17(11), 1/20–24(9), 1/26–30(10)	40	70—1944	

^a 10 semi-evergreen seedlings, terminals remained evergreen, and 1 evergreen seedling.

the bushy habit. This expectation was realized and some of these bushy seedlings, when combined with good fruit and flower characters, may prove to be of value for backyard plantings.

(b) *Evergreen vs. deciduous habit.*—A small F_2 population of Chinese Dwarf Evergreen x Rio Oso Gem, grown in the Horticulture Orchard of the College of Agriculture at West Los Angeles, only 7 miles from the ocean, segregated into five deciduous to six semi-evergreen to five evergreen. The five seedlings classed as evergreen showed no bare areas in the winter of 1942 and the terminal shoots continued growing. The trees classed as semi-evergreen varied in behavior, three trees merely retaining their terminal leaves, all the rest of the branches dropping them, while the terminals of the other three semi-evergreen trees continued growth but most of the rest of the tree dropped its leaves. These sixteen trees are the only survivors of 41 embryos cultured in the fall of 1940, the loss being due to the adverse effect of low greenhouse temperature. Since there is evidence of differential survival in favor of low chilling and evergreen types when embryo-cultured seed-

lings are grown at temperatures of 45–55° (Lammerts, 1943), it is impossible to draw conclusions from this population as to the number of factors involved in deciduous vs. evergreen behavior. A much larger F_2 population from the same cross, grown and observed at the Armstrong Nurseries, Ontario, California, 50 miles from the ocean where winters are colder than in West Los Angeles, segregated into fifty-nine deciduous to ten partial evergreen and only one evergreen. Evidently, then, the evergreen character is recessive but due to the interaction of several factors, which are inhibited in their expression by low temperatures. Even at the West Los Angeles Station, the trees originally classed as evergreen cast most of their leaves toward the base of the tree in the winter of 1943 and 1944 when they were three and four years old respectively. The evergreen behavior is then not so strongly expressed in the older trees, a characteristic observed in the Chinese Dwarf Evergreen but not so noticeable because of the very short branches. St. Helena trees now three years old seem to be more truly evergreen in their behavior than the evergreen derivatives of Chi-

nese Dwarf Evergreen so far observed at West Los Angeles.

(c) *Short vs. long chilling requirement.*—In table 2 the leafing out dates of the various F_2 progenies obtained by selfing indicated plants of the F_1 hybrids shown in table 1 are summarized. Seedlings were recorded as beginning to leaf out when from one-eighth to one-quarter inch of leaf growth had occurred on at least the terminal and most of the lateral buds. As shown by Lammerts (1941) this method of indexing varieties as to chilling requirement is more accurate than the use of arbitrary grade numbers. Though insufficient chilling results in many other effects, delayed foliation is the most easily measurable one so far observed. In spite of the fact that different years are involved in the recording of the F_1 and F_2 data, it is believed they are comparable because all of these years were relatively mild. Even in 1942 the chilling requirement of varieties such as Rio Oso Gem was far from completely satisfied, it being delayed in leafing until April 15. Following the relatively severe winter of 1936-37 it leafed out March 9. Furthermore two different F_2 progenies from the same F_1 hybrid, 36058/2, one recorded in 1941 following a very mild winter, and the other in 1942, both show the same range in leafing out dates. Also trees recorded in 1938 and 1939 maintained the same sequence of leafing out during the rest of the period covered by the records presented in table 2. It was observed that as the seedlings became older their chilling requirement increased slightly. Several facts are clearly shown by a study of the data presented. These may be summarized as follows:

(1) All F_1 hybrids without exception show an F_2 segregation characterized by the appearance of seedlings definitely much earlier leafing than the F_1 parents used.

(2) F_2 progenies of the relatively late leafing F_1 hybrids such as 36058/11, 36057/17, and 36057/22 have seedlings which are just as early leafing as those found in progenies of the early leafing F_1 s such as 36058/2.

(3) The latest leafing F_2 seedlings are but rarely significantly later leafing than their F_1 parents. Even in the case of the Chinese Dwarf Evergreen x Rio Oso Gem, only five of the F_2 seedlings were later leafing than the F_1 . None of the F_2 seedlings was as severely delayed as even young trees of Rio Oso Gem.

(4) By selfing varieties such as Babcock and even Rio Oso Gem which is usually very severely delayed in leafing in Southern California, seedlings which are much earlier leafing may readily be obtained. In fact, several promising new varieties have been obtained in this way.

(5) St. Helena, however, when close pollinated, gave 43 evergreen and one partially evergreen seedling, all of which were very early leafing. The spread of 20 days in leafing out of the lateral buds was relatively small, though obviously this variety is not

homozygous for all the early leafing or low chilling factors.

(6) Some of the earliest leafing F_2 seedlings obtained from Babcock x Early Double Pink, both deciduous, retained their leaves so late in the winter and leafed out so early in the spring as to be very similar in their behavior to the partially evergreen seedlings found in the F_2 of Chinese Dwarf Evergreen x Rio Oso Gem. This would seem to indicate that evergreen behavior may merely be due to the accumulation of a series of recessive factors causing a very low chilling requirement. On the other hand the lateral buds of some evergreen and semi-evergreen trees were later in leafing out than those of the earliest leafing deciduous sibs, though all were relatively early leafing.

The above facts indicate that a low chilling requirement is due to the accumulation of many factors, most of which are recessive and cumulative. However, some recessive factors causing a high chilling requirement are also involved by the F_2 population of Chinese Dwarf Evergreen x Rio Oso Gem where five seedlings having a significantly longer chilling requirement than the F_1 hybrid occurred. More complete analysis of the factors involved in the inheritance of low vs. high chilling requirement as well as their relation to evergreen behavior awaits the development of lines breeding true for (1) evergreen behavior, (2) low chilling deciduous and (3) high chilling deciduous behavior. Intercrossing of such strains, selfing of their F_1 hybrids and backcrossing to appropriate tester lines should give a much clearer picture of the obviously complicated factorial relationship.

(2) *Flower characters.*—*Size.*—As reported by Conners (1919, 1922), Blake (1931) and Bailey and French (1942) there is a very distinct break between the recessive, large, broad petaled type called the "showy" flowered class by Blake (1931) and the dominant, medium or narrow petaled, "non-showy" class. All the ornamental varieties used have double flowers of the showy type. The following varieties when intercrossed give all showy flowers in the F_1 ; Chinese Dwarf Mandarin or Evergreen, Early Double Pink, Early Double Red, Peppermint Stick, Rio Oso Gem, Vainquer, Mayflower, Quetta Nectarine, Goldmine Nectarine and Lukens Honey. They all, therefore, have the same recessive sh factor in common. Sims, Early Imperial and Miller's Late carry the homozygous, dominant, non-showy factors ShSh, as they give all non-showy flowers when crossed to the above varieties. Elberta as reported by Bailey and French (1942), July Elberta, and Socala are heterozygous, as they give 1:1 ratios for showy vs. non-showy when crossed to Lukens Honey. Selfed progenies of showy x non-showy gave clear-cut ratios of 3 non-showy:1 showy.

As reported by Bailey and French (1942) variation in the non-showy class occurs so that the heterozygous, medium-flowered genotype cannot be readily separated from the homozygous, small-flowered

TABLE 3. *Classification of backcross progenies as to color and petal number.*

Grade	Charac- teristic number of petals	Amount of variation	Flower color	F ₁ (Babcock x Early Double Red) x Early Double Red 18-24 petals			36058/11 (Babcock x Early Double Pink) x Early Double Pink 10-15 petals
				36057/4	36057/19	Total	
1	5	Very rarely extra petal or petaloid	Red	8	5	13	..
			Pink	7	7	14	23
2	5	Appr. 10% flowers 1-3 extra petals or petaloids	Red
			Pink	3
3	5	Appr. 25% flowers 1-3 extra petals or petaloids	Red
			Pink	4
4	5	Appr. 50% flowers 1-3 extra petals or 1-6 petaloids	Red
			Pink	5
5	5-10	Often 2-3 extra petaloids. 5 petal flowers rare	Red
			Pink	2
6	10-14	Rarely flowers with only 5 petals	Red
			Pink	2
7	10-14	Rarely flowers with 5+1-3 petaloids	Red	2	..	2	..
			Pink	1	..	1	2
8	14-16	Occasional extra petaloids. No flowers with only 5 petals	Red	5	3	8	..
			Pink	3	2	5	7
9	15-18	Occasional extra petaloids. No flowers with only 5 petals	Red	1	..	1	..
			Pink	..	1	1	..
10	15-20	Occasional extra petaloids. No flowers with only 5 petals	Red	1	1	2	..
			Pink	1	1	2	..
11	20-24	Occasional extra petaloids. No flowers with only 5 petals	Red	1	..	1	..
			Pink	1	2	3	..
		Total all classes	Red	18	9	27	48
			Pink	13	13	26	

type. In the showy class two distinct flower size types occur, the small showy such as Babcock and Chinese Dwarf Evergreen which range from 14 to 17 millimeters long by 11 to 13 mm. wide, and the large showy type such as Early Double Pink or Red, Rio Oso Gem, Mayflower, Lukens Honey and many of the nectarines such as Goldmine, ranging from 21 to 30 mm. long by 15 to 19 mm. wide. Some variation in this large-flowered group occurs in that the range in Lukens Honey is 21 to 22 mm. long by 15 to 18 mm. wide, whereas Peppermint Stick ranges from 23 to 30 mm. long by 15 to 19 mm. wide. All the F₁ hybrids of Babcock x Early Double Red had flowers larger than those of Babcock. In the backcross to Early Double Red, 14 trees had flowers about the size of the F₁ flower and 17 had definitely larger flowers comparable in size to those of Early Double Red. Though there was some variation in both classes, there is one main gene L for large flower size which is incompletely dominant. The effect of this gene on the size of the non-showy flower was not studied.

It is fortunate from the breeding point of view that both Socala and July Elberta carry the recessive sh factor, thus making it possible, by selfing showy flowered F₁ hybrids, to get all showy flowered F₂ generations from which selections based on the

degree of doubleness or fruit quality may be made.

Flower color.—Ornamental flowering peaches may be either light or dark pink, red or white. Many varieties varying in petal number and chilling requirement occur in each of these color classes. The most strikingly beautiful variety, however, is the variegated red, pink and white Peppermint Stick with very large flowers having from 17 to 25 petals. As shown in table 3, clear-cut segregation of pink and red in a 1:1 ratio occurred in the progeny obtained by backcrossing Babcock x Early Double Red to Early Double Red. The F₂ population (table 4) segregated in the ratio of 3 pink:1 red. Red flower color may then be denoted by the recessive symbol rr and pink by the symbol RR. The flowers of Early Double Pink are a darker shade of pink than those of Babcock or Rio Oso Gem. The F₁ hybrid flowers were also slightly darker. Several F₂ progenies were classified as to shade of pink, placing all the trees with flowers as dark as the F₁ or darker in one class and those with the light pink Babcock type of flower in the other. These populations segregated as shown in table 5.

Evidently light pink is recessive to dark pink and is due to one main recessive factor, so may be denoted by the symbol pp, dark pink being PP. The

TABLE 4. Classification of F₂ progenies as to color and petal number.

Grade	Characteristic number of petals	Amount of variation	Flower color	F ₁ trees of Babcock x Early Double Red										F ₁ trees of Babcock x Early Double Pink									
				5 petals					18-24 petals					5 petals					10-15 petals				
				36057/4—1938	36057/4—1939	36057/14—1938	36057/14—1939	36057/17—1939	36057/19—1939	36057/22—1938	36057/23—1938	36057/30—1938	36057/42—1938	Total	36058/2—1938	36058/2—1939	36058/11—1939	36058/16—1938	36058/28—1939	36058/33—1938	36058/33—1939	Total	
1	5	Very rarely extra petal or petaloid	Red	5	9	3	2	9	3	..	4	1	3	39	1	6	7	
			Pink	10	33	5	3	22	3	6	10	5	1	98	5	25	35	6	18	5	9	103	
2	5	Appr. 10% flowers 1-3 extra petals or petaloids	Red	..	1	1	1	
			Pink	..	2	1	3	..	1	2	..	1	1	2	7	
3	5	Appr. 25% flowers 1-3 extra petals or petaloids	Red	1	1	
			Pink	3	..	3	6	
4	5	Appr. 50% flowers 1-3 extra petals or 1-6 petaloids	Red	..	1	..	1	2	
			Pink	2	2	1	..	3	..	1	..	2	7	
5	5-10	Often 2-3 extra petaloids. 5-petal flowers rare	Red	
			Pink	1	1	1	..	1	4	2	1	3	
6	10-14	Rarely flowers with only 5 petals	Red	2	1	1	4	
			Pink	3	3	1	..	7	2	2	
7	10-14	Rarely flowers with 5 plus 1-3 petaloids	Red	..	1	1	2	
			Pink	..	1	1	2	..	1	2	..	2	..	1	6	
8	14-16	Occasional extra petaloids. No flowers with only 5 petals	Red	1	1	2	
			Pink	..	3	3	1	2	9	
9	15-18	Occasional extra petaloids. No flowers with only 5 petals	Red	1	1	
			Pink	1	1	
10	15-20	Occasional extra petaloids. No flowers with only 5 petals	Red	1	1	
			Pink	1	1	
11	20-24	Occasional extra petaloids. No flowers with only 5 petals	Red	
			Pink	
		Total—All classes of petal number	Red	7	13	4	3	11	4	..	5	1	4	52	1	8	9	
			Pink	14	43	8	3	25	7	9	11	6	1	127	6	30	44	8	26	6	14	134	

three colors so far discussed are then constituted as follows:
Dark Pink PPRR, PpRR, PpRr
Light Pink ppRR, ppRr
Red PPrr, Pprr, pprr

Thus, only dark pink flowers are obtained by crossing Babcock (ppRR) x Early Double Red (PPrr), but in the F₂ segregation occurs in the ratio of 9 dark pink to 3 light pink to 4 red. The Early Double Pink clone used in these experiments was then of the constitution PPRr, since one of the F₁ hybrids, tree 36058/2, segregated in the ratio of 30 pink to 8 red (see table 4). As mentioned above, Peppermint Stick is variegated with white as the continuous phase. In appearance it is similar to the variegated *Rhododendron obtusum* described and illustrated in color by Imai (1937). Most of the flowers are white with red, dark pink and light pink streaks and flecks. Some of the larger dark pink areas have light pink streaks. Some flowers are light or dark pink with red streaks. Often entirely red flowers and sometimes even whole

TABLE 5. Color segregation observed in F₂ populations of Babcock x Early Double Pink and Red.

	Dark Pink	Light Pink	Red	Total
Babcock x Early Double Red selfed				
Population 38023	11	3	7	21
38025	9	2	5	16
39031	19	6	11	36
Babcock x Early Double Pink selfed				
39023	25	5	8	38
Total all populations.....	64	16	31	111
Expected 9:3:4 ratio.....	63.5	20.8	27.7	111

TABLE 6. Summary of segregation as to petal number observed in backcross of Babcock x Early Double Pink to Early Double Pink.

Flower type	Singles	1-5 extra petals grades 2-5	10-16 petals grades 6-8	Total
Genotypes	D_1d_1 Dm_1dm_1 and D_1d_1 dm_1dm_1	d_1d_1 Dm_1dm_1	d_1d_1 dm_1dm_1	
Observed frequency	23	14	11	48
Expected frequency	24	12	12	48

branchlets with only red flowers occur. Completely white flowers are less common and entirely white flowered branchlets are very rare. The bark on the young branchlets is without anthocyanin pigment except for streaks which are usually very narrow. This variety was crossed to Rio Oso Gem in hopes of developing a variegated variety with good quality yellow fruit. The F_1 hybrids were all large flowered single dark pink. The F_2 segregated into 32 pink to 8 red to 5 variegated to 6 white. All the variegated plants were genetically white as shown by their bark, which like that of white, had no anthocyanin pigment except for occasional narrow streaks. The flowers of the variegated plants all had white as the continuous phase, but some were pink, red and white, while others were merely pink and white variegated. This is to be expected since the variegated plants would be either genetically red or pink. Variation in the degree of variegation also occurred, some plants having most of the flowers entirely white or with only a narrow stripe on one or two petals. Since plants variegated for pink and red only were not found, it would seem that the recessive variegation factor can only express itself in the presence of a genetically white background. Its behavior is thus comparable to other variegation factors reported (Imai, 1937) and so is probably allelomorphic to white and may be represented by the symbol w^v . The trees classified as white would then according to this hypothesis be either reversions to white or more probably low grade variegated since some of the young trees with only a few white flowers may when older show some variegated flowers. Considering the variegated and white plants together, we have the following distribution:

	Pink	Red	White and variegated
Actual distribution	32	8	11
Expectation 9:3:4 ratio	28.69	9.56	12.75

White is then recessive to colored, and may be denoted by the symbols ww , and colored by WW . Though it is clear that the variegation factor may only express itself in the presence of the ww factors, the exact mechanism of its expression is not known.

Petal number.—The flowers of Early Double Pink have from 12 to 15 petals, and those of Early

Double Red from 18 to 24 petals. A variable number of petaloids, usually not more than three, may also occur. The F_1 hybrids have single flowers with only five petals. In tables 3 and 4 the trees of the backcross and F_2 populations are classified according to the degree or grade of doubleness displayed. Those in any one of the lower grades distinctly differed from those in any other grade in the percentage of flowers with extra petals, while in the higher grades where no single flowers occurred, classification was based on distinct differences in the average number of petals and range of variation. Thus, trees in grade 9 rarely had as many as 18 petals while those in grade 10 usually had 18-20 petals but occasionally had only 15 petals.

The segregation occurring in the backcross to Double Pink (table 3) indicates that Early Double Pink differs from Babcock in at least two recessive factors, one of which is only able to affect petal number when the other is homozygous. The main or basic factor for singleness may be denoted by the symbol D_1 . This factor is almost completely dominant, only occasional extra petals being formed in D_1d_1 plants, and even when heterozygous is able to inhibit any expression of doubleness by the other or modifying factor which may be denoted by the symbol dm_1 . Thus, Babcock is D_1D_1 Dm_1Dm_1 , the Early Double Pink is d_1d_1 dm_1dm_1 , and the F_1 is D_1d_1 Dm_1dm_1 . The segregation shown in table 3 is summarized in table 6. Grades 2-5 in the group with 1-5 extra petals are probably due to minor modifying factors.

The segregation obtained by backcrossing to Early Double Red shows that this variety has a second modifying factor, dm_2 , which increases the number of petals to as many as 20-24, though occasionally flowers with as few as 15 petals may occur on young trees. The segregation shown in table 3 is summarized in table 7.

As the trees in this backcross received all three factors for doubleness from the pollen parent instead of only two, the degree of doubleness was higher, that is, no trees occurred in grades 2-6. The genotype d_1d_1 Dm_1dm_1 Dm_2dm_2 is then more double than d_1d_1 Dm_1dm_1 Dm_2Dm_2 , which indicates that Dm_2 is not completely dominant. Similarly, d_1d_1 dm_1dm_1 Dm_2dm_2 is more double than d_1d_1 dm_1dm_1 Dm_2Dm_2 . The observed segregation fits the trifactorial analysis very closely.

TABLE 7. Summary of segregation as to petal number observed in backcross of Babcock x Early Double Red to Early Double Red.

Flower type	Singles			10-18 petals grades 7-9			15-24 petals grades 10-11			Total
	D ₁ d ₁	Dm ₁ dm ₁	Dm ₂ dm ₂	d ₁ d ₁	Dm ₁ dm ₁	Dm ₂ dm ₂	d ₁ d ₁	dm ₁ dm ₁	dm ₂ dm ₂	
Genotype	D ₁ d ₁	dm ₁ dm ₁	Dm ₂ dm ₂	d ₁ d ₁	Dm ₁ dm ₁	dm ₂ dm ₂				
	D ₁ d ₁	Dm ₁ dm ₁	dm ₂ dm ₂	d ₁ d ₁	dm ₁ dm ₁	Dm ₂ dm ₂				
	D ₁ d ₁	dm ₁ dm ₁	dm ₂ dm ₂							
Observed frequency	27			18			8			53
Expected frequency	26.5			19.9			6.6			53

The F₂ populations also fit the above analysis very well. Thus, in the F₂ of Babcock x Early Double Pink, one would expect three-fourths of the trees to carry the D factor and so to be singles, and one-fourth to have the dd factors and exhibit varying degrees of doubleness. Among the doubles one-fourth should be as double as Early Double Pink, i.e., have 10-15 petals. As may be seen from table 8, where the data presented in table 4 are summarized, this was actually the case.

In the F₂ of Babcock x Early Double Red, three-fourths of the population would again be expected to carry the D factor and so to be singles, and one-fourth should have the d factor and show varying degrees of doubleness. Among the doubles one-sixteenth should be as double as the pollen parent, that is, have 18-24 petals. As may be seen from table 9, where the data presented in table 2 are summarized, this was the case.

The two trees in grade 10 did not have flowers with quite as many petals as the maximum number found in Early Double Red. Probably this was due to the youth of the trees, as certain other trees saved for backcrossing tended to show a wider range in petal number when older. As in the backcross population, the degree of doubleness was greater than in the F₂ of Babcock x Early Double Pink. Thus, only 3/64 or 8.4 trees would be expected to have the constitution d₁d₁ Dm₁dm₁ Dm₂dm₂ or d₁d₁ Dm₁dm₁ Dm₂dm₂ and so have flowers in grades 2-5. Actually, 12 trees were classified into these grades, as compared to 22 trees in the smaller F₂ population of Babcock x Early Double Pink.

DISCUSSION.—Consideration of the data presented indicates that the problems involved in breeding edible ornamental flowering peaches are fairly complex. Thus, at least nine factors are involved when breeding for color, petal number and size, alone. A good ornamental peach should furthermore be de-

ciduous and have a chilling requirement short enough to flower uniformly and abundantly following the mildest winters, yet long enough to prevent leafing out until after the flowering peak is past. Thus, a chilling requirement as short as that of St. Helena would not be desirable, as too much leaf development would detract from the beauty of the flowering sprays. Resistance to peach leaf curl is important when planting in the coastal areas, since backyard trees are rarely sprayed.

Fortunately large petal size and dark pink flower color are both dependent upon dominant factors. One may also either use only the showy type of commercial varieties or eliminate the non-showy seedlings by selecting showy F₁ hybrids. As regards flower size, color and petal number, it is best to use Early Double Red as the pollen parent in crossing with commercial varieties, since it is homozygous for the dark pink factor, the large flower size factor and all three factors for doubleness, d₁d₁ dm₁dm₁ dm₂dm₂. Accordingly, more F₂ trees with flowers double enough to be desirable as ornamentals will be obtained than when varieties with fewer petals, such as Early Double Pink, are used.

The extra petals in most varieties of ornamental peaches do not arise from conversion of stamens into petals and petaloids, since the flowers of even Early Double Red have just as many stamens as a single flowered peach, that is, 40-45 stamens. The Clara Meyer flowering peach seems to be a genetically distinct type, for it has 80-90 petals, only 10-30 anthers, and a calyx with thickened lobes and 5 extra smaller lobes. The Helen Borchers variety may also be genetically distinct, for it has from 30-40 petals and 5 extra calyx lobes. The genetic basis for doubleness in these varieties should be determined. Though very beautiful in Northern California, their chilling requirement is too long for satisfaction by our mild winters and so new varieties, combining their high

TABLE 8. Summary of segregation as to petal number observed in F₂ progenies of Babcock x Early Double Pink.

Flower type	Singles Grades 1	Semi-doubles Grades 2-5	Doubles similar to Early Double Pink Grades 6-7	Total
Genotype	D factor present	D factor absent	d ₁ d ₁ dm ₁ dm ₁	
Observed frequency	110	25	8	143
Expected frequency	107.3	26.8	8.9	143

TABLE 9. Summary of segregation as to petal number observed in F_2 progenies of Babcock x Early Double Red.

Flower type	Singles Grade 1	Semi-doubles Grades 2-9	Doubles similar to Early Double Red Grades 10-11	Total
Genotype	D factor present	D factor absent	d_1d_1 dm_1dm_1 dm_2dm_2	
Observed frequency	137	40	2	179
Expected frequency	134.3	41.9	2.8	179

degrees of doubleness with a shorter chilling requirement, are needed. The same situation obtains as regards existing white varieties.

Peppermint Stick, like Early Double Red, transmits all the doubleness and large size factors and in addition, transmits its variegation factor to about half of the genetically white seedlings. It should therefore be possible to develop variegated, yellow fleshed peaches of good quality by selection and intercrossing of selected F_2 seedlings from Peppermint Stick x Rio Oso Gem.

SUMMARY

(1) As a result of analysis of F_1 and F_2 progenies obtained by crossing the ornamental flowering Early Double Pink, Red, Peppermint Stick and Chinese Dwarf Evergreen with the commercial varieties Babcock, Rio Oso Gem, and Socala, eleven factors have been established in the peach as follows:

A recessive factor dw causes a very dwarf habit, seedlings homozygous for this factor growing to a height of only 1-1½ feet as compared with 5-6 feet for the normal type in a two-year period.

Seedlings homozygous for the duplicate bu_1 bu_2 factors are semi-dwarf bushy trees growing only to a height of 2½-3 feet in two years. Either factor acting alone showed no appreciable effect.

L is a semi-dominant factor for large flower size in the showy (sh) flowered class.

As regards flower color, there is a recessive r factor for red flowers, a recessive p factor for light

pink and a recessive w factor for white flowers. Flowers, in order to be colored at all, must have at least one dominant W factor. A variegation factor, w^v , allelomorphous to white, is able to cause variegation for red, pink and white only when the plants are of the rr constitution, plants which are Rr or RR being pink and white variegated.

Three factors, d_1 completely recessive, and dm_1 dm_2 incompletely recessive, determine the degree of doubleness. Plants with d_1d_1 have only 1-5 extra petals; d_1d_1 dm_1dm_1 plants have 10-16 petals as in Early Double Pink, and d_1d_1 dm_1dm_1 dm_2dm_2 plants have 15-24 petals as in Early Double Red and Peppermint Stick.

(2) The ornamental flowering peach varieties have both the showy (sh) factor established by Blake and the L factor, in addition to being of various grades of doubleness.

(3) Evergreen habit is recessive but dependent on the interaction of several factors which are limited in their expression by low relative temperatures.

(4) Low chilling requirement is due to the cumulative effect of a series of recessive factors and probably several semi-dominant ones.

(5) The bearing which these factors have on the problem of breeding ornamental flowering peaches of good quality is discussed.

DIVISION OF HORTICULTURE,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES, CALIFORNIA

LITERATURE CITED

- BAILEY, J. S., AND A. P. FRENCH. 1942. The inheritance of blossom type and blossom size in the peach. *Proc. Amer. Soc. Hort. Sci.*, 40: 248-250.
- BLAKE, M. A. 1931. Flower types developed by peach seedlings. *New Jersey Agric. Exp. Sta. Rep.* 52: 258-259.
- . 1933. Additional facts in regard to the J. H. Hale peach as a parent in breeding work. *Proc. Amer. Soc. Hort. Sci.* 30: 124-128.
- CONNERS, C. H. 1919. Some notes on the inheritance of unit characters in the peach. *Proc. Amer. Soc. Hort. Sci.* 16: 24-36.
- . 1922. Peach breeding: A summary of results. *Proc. Amer. Soc. Hort. Sci.* 19: 108-115.
- IMAI, YOSHITAKA. 1937. Bud variation in a flaked strain of *Rhododendron obtusum*. *Jour. College of Agric. Tokyo Imperial University* 14: 93-98.
- LAMMERTS, W. E. 1941. An evaluation of peach and Nectarine varieties in terms of winter chilling requirements and breeding possibilities. *Amer. Soc. Hort. Sci.* 39: 205-211.
- . 1942. Embryo culture an effective technique for shortening the breeding cycle of deciduous trees and increasing germination of hybrid seed. *Amer. Jour. Bot.* 29: 166-171.
- . 1943. Effect of photoperiod and temperature on growth of embryo cultured peach seedlings. *Amer. Jour. Bot.* 30: 707-711.

APPARENT LOCALIZATION OF FUSARIUM WILT RESISTANCE IN THE PAN AMERICA TOMATO ¹

P. H. Heinze and C. F. Andrus

THE HIGH degree of resistance to the wilt disease caused by *Fusarium oxysporum f. lycopersici* Snyder and Hansen, in the tomato variety Pan America introduced by Porte and Walker (1941), has been proved by many laboratory and field tests. The near immunity is equal to that of the most resistant lines of *Lycopersicon pimpinellifolium* (Jusl.) Mill., the wild host which was used as a parent of Pan America.

Grafting has been used to study the nature of disease resistance in several plants (Bond, 1936; Leach, 1929; May, 1930; and Schmidt, 1933), but in the experiments dealing with wilt resistance in tomatoes the varieties used as resistant are now recognized as having a much lower degree of resistance than Pan America. In the experiments reported here Bonny Best, a variety long recognized as very susceptible to the tomato wilt fungus, and the resistant Pan America variety were used. *L. pimpinellifolium* plants were used in one experiment.

MATERIALS AND METHODS.—The seeds were planted in flats of one-half composted soil and one-half sand mixture. After the cotyledons were fully expanded the seedlings were transplanted to 2½-inch pots. When the plants were about three weeks old they were used for grafting. Two types of grafts were used—stem approach grafts and stem splice grafts. The approach grafts were made by removing a thin section about one inch in length, between the cotyledons and the first leaf, from adjacent sides of the two stems. The cut surfaces were immediately placed against each other and securely wrapped with a rubberized paraffin tape known as Parafilm (fig. 1). The graft components were both left intact until the plants were examined for wilt. The splice grafts were made by severing the two plants with a smooth slant-

ing cut about one inch in length between the cotyledons and the first leaf. The scions of the two plants to be grafted were exchanged, put into place and wrapped with Parafilm (fig. 2). The cuts were made with a wet razor and the surfaces kept moist until the grafting operation was completed. The splice grafted plants were placed in a moist chamber for three to five days, then removed and inoculated. All plants were inoculated according to the procedure described by Wellman (1939), and planted on a greenhouse bench. Two or three weeks after inoculation the plants were examined for external and internal symptoms of disease. External symptoms were evidenced by wilting, curling or yellowing of the leaves, stunting, and frequently, death of the plant. Internal symptoms were determined by cutting through the vascular tissues and noting the presence of discoloration in the bundles. Unless the discoloration was easily detected and extended above the soil level, the plants were considered as showing slight symptoms, or as being free, and were so classified.

The plants in these experiments were necessarily somewhat older, due to the procedure of grafting, than those ordinarily used in wilt resistance tests. The greater age may have been responsible for a slightly slower rate of wilting and the occurrence of an occasional escape, but the development of the disease was entirely satisfactory for the purpose of this study.

RESULTS.—*Approach grafts.*—Three experiments were carried out involving approach grafts of Bonny Best and Pan America plants. The plants of the first experiment, grafted in October, 1942, and those of the second experiment grafted in December, were examined two weeks after inoculation; the plants in the third experiment were grafted in December and examined three weeks after inoculation. The results are given in table 1. In experiments 1 and 2 some

¹ Received for publication August 28, 1944.
Contribution No. 44, U. S. Regional Vegetable Breeding Laboratory, Charleston, South Carolina.

TABLE 1. Effects of approach grafting on infection of tomato plants by *Fusarium*.

Experiment number ^a	Varietal component	Total number of plants	Symptoms of infection			Examined days after inoculation
			External	Internal only	Slight or none	
1	Bonny Best—	50	35	5	10	14
	Pan America	50	4	19	27	14
	Bonny Best, ungrafted	25	23	2	0	14
	Pan America, ungrafted	24	1	1	22	14
2	Bonny Best—	48	34	12	2	14
	Pan America	48	6	5	37	14
3	Bonny Best—	44	41	3	0	21
	Pan America	44	4	20	20	21
	Bonny Best, ungrafted	24	22	2	0	21
	Pan America, ungrafted	26	0	1	25	21

^a Plants in experiment 1 were grafted on 10/30/42, all others were grafted on 12/28/42.

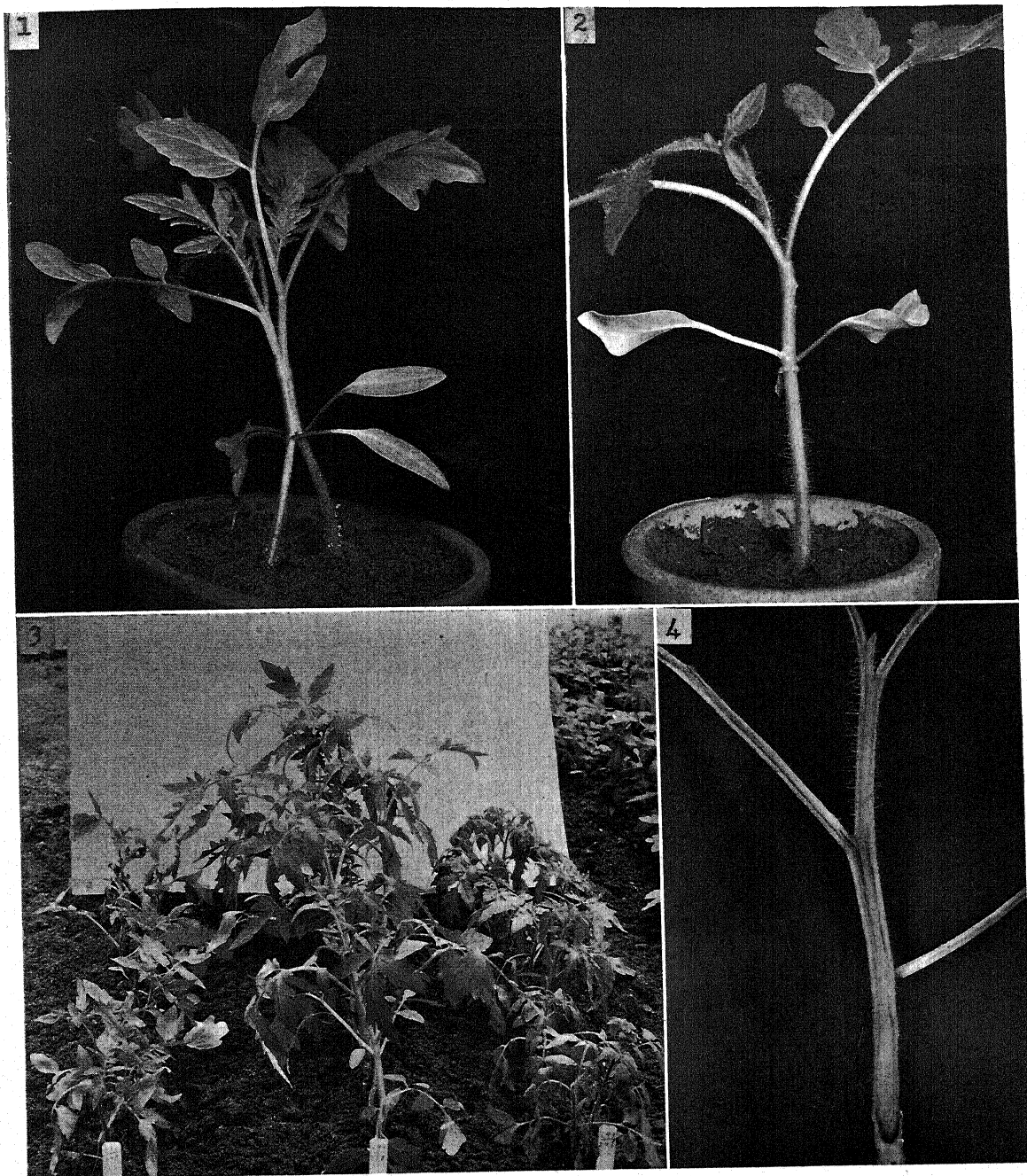


Fig. 1-4.—Fig. 1. Newly completed approach graft.—Fig. 2. Newly completed splice graft.—Fig. 3. Splice grafted plants two weeks after inoculations. Left, Pan America scion on Bonny Best stock; center, Bonny Best scion on Pan America stock; right, Bonny Best scion on Bonny Best stock. —Fig. 4. Sectional view of a Pan America scion on a Bonny Best stock two weeks after inoculation showing the extent of the discoloration of the vascular tissue.

of the Bonny Best components escaped infection but in experiment 3, where the plants were allowed to grow three weeks before examination, infection was found in all Bonny Best plants. All of the ungrafted Bonny Best control plants showed infection in the approach-grafting experiments.

In the three experiments a total of 44 Pan America components showed only internal symptoms. Of

the 44 plants, 22 had symptoms above the graft union but not below, whereas only three showed symptoms below the union and not above. Nineteen plants had symptoms both above and below the graft union. In nearly all cases where symptoms were found below the graft union the vascular discoloration appeared to extend downward from the region of the union.

TABLE 2. *Effects of splice grafts on infection of tomato plants by Fusarium.*

Varietal components	Total number of plants	Symptoms of infection		
		External	Internal only	Slight or none
Bonny Best scion	121	4	4	113
Pan America stock				
Pan America scion	120	118	2	0
Bonny Best stock				
Bonny Best scion	99	95	3	1
Bonny Best stock				
Pan America scion	97	1	3	93
Pan America stock				
Bonny Best, ungrafted	85	77	4	4
Pan America, ungrafted	85	5	3	77

Splice grafts.—On November 18 and on each of the two succeeding days 25 grafts of each of the following combinations were made: Bonny Best scion on Pan America stock, Pan America scion on Bonny Best stock, Bonny Best scion on Bonny Best stock, and Pan America scion on Pan America stock. The plants were examined fourteen days after inoculation and the resistance to infection noted. In January another set of grafts involving the same kinds of plants and the same types of grafts were made. These were also examined fourteen days after inoculation. The combined results of these experiments are presented in table 2. Among the 121 Bonny Best scions on Pan America stocks slight external symptoms were discernible on only four. Four other plants had some internal darkening of the vascular tissue and in each case this occurred below the graft union. The Pan America scions on Bonny Best stocks showed severe wilt symptoms in 118 out of a total of 120 plants. The other two plants showed internal symptoms both above and below the graft union. All but one of the Bonny Best scions on Bonny Best stocks showed symptoms of wilt. Four of the 97 Pan America scions on Pan America stocks showed some wilt symptoms. A few susceptible Pan America plants appeared in nearly all of these experiments but they usually represented less than five per cent of the total number. The Pan America scions on Bonny Best stocks showed more severe symptoms of wilting, stunting and yellowing of the leaves than the Bonny Best scions on Bonny Best stocks (fig. 3). The darkening of the vascular tissue extended well up into the Pan America scion (fig. 4). A number of isolation tests showed that the organism was present in these scions. When the grafts involving Bonny Best scions on Bonny Best stocks and Pan America scions on Bonny Best stocks were made and the plants allowed to grow without inoculation, all grew readily and no discernible differences could be detected in the vigor of the two lots.

A few Bonny Best scions were grafted on to *L. pimpinellifolium* stocks and the reciprocal grafts made. These plants did not grow as well as the

grafted Bonny Best-Pan America plants. The buds in the axils of the cotyledons grew from a majority of both the Bonny Best and *L. pimpinellifolium* stocks and formed branches nearly as large as the grafted scion. The results of the inoculations were essentially the same as the Bonny Best-Pan America grafts. Ten of the 11 Bonny Best scions on *L. pimpinellifolium* stocks were free of wilt and all of the 12 *L. pimpinellifolium* scions on Bonny Best stocks had pronounced wilt symptoms.

In October, 1948, a number of splice grafts of Pan America scions on Bonny Best stocks, Bonny Best scions on Bonny Best stocks, and Bonny Best scions on Pan America stocks were made. After the grafted plants were removed from the moist chambers they were placed on a sand bench in a slanting position and a portion of the stem above the graft union was injured slightly and covered with sand to produce adventitious roots. When the roots were several inches long (after 14 to 18 days) the plants were inoculated through the adventitious roots. The whole plant was then transferred to another bench containing soil. The pot containing the original uninoculated root system of the stock was partially covered with soil and the adventitious roots were also covered. Of the 29 Pan America scions on Bonny Best stocks only one showed slight internal symptoms. The other 28 were free of disease. Twenty-three of the 32 Bonny Best scions on Bonny Best stocks showed definite external symptoms, seven showed internal symptoms, and two were apparently free of disease. Of the 45 Bonny Best scions on Pan America stocks, 25 showed external symptoms, 12 had internal symptoms, and 8 were apparently free of disease. In general, the last group, Bonny Best scions on Pan America stocks, seemed to be somewhat more vigorous than the Bonny Best scions on Bonny Best stocks.

Cuttings were also made from the tops of some Bonny Best and Pan America plants and allowed to root on a sand bench, then inoculated through the new roots. The Bonny Best plants showed the same susceptible reaction and the Pan America plants the

same resistant properties as when the original root systems were used.

Culture experiments.—A few experiments were conducted to determine if an extract or sterilized tissue from the Pan America plant would inhibit the growth of the tomato wilt fungus. Tissues from the above-ground portion of each of the varieties, Pan America and Bonny Best, were ground in a Nixtamal mill and the juices pressed out by use of a hydraulic press. The juice was centrifuged and filtered through a No. 4 sintered glass filter. Five milliliters of the juice extract were run into a petri dish with 10 ml. of two per cent potato dextrose agar. As soon as the agar had cooled the medium was inoculated with the tomato wilt fungus. Extracts were made from the roots of each variety in a similar manner, but the *Fusarium* grew equally well on all extracts.

Ten plants, twelve to eighteen inches tall, from each variety, were cut into three-inch sections. Each plant yielded four or five such sections, the first representing most of the underground portion of the plant with the branch roots removed and each succeeding section a higher position on the stem. All sections were placed in individual test tubes, sterilized in an autoclave, cooled and inoculated. The tomato wilt fungus grew well on all sections, with no perceptible difference between varieties or positions of the section on the plant. No difference between varieties could be detected in the growth of the *Fusarium* when the whole root systems were placed in liquid media, sterilized in an autoclave and inoculated. The liquid medium was prepared as described by Wellman (1939).

Sterilization of tissues without the use of heat, i.e., by using mercuric chloride, did not prove satisfactory for culture experiments.

DISCUSSION.—When the susceptible Bonny Best plants were approach-grafted to resistant Pan America plants the wilt organism crossed the graft union from the Bonny Best to the Pan America in about 40 per cent of the plants and tended to move upward in the Pan America stems much more freely than downward. In the first experiments there was an indication that the Bonny Best plants were receiving some degree of resistance from their Pan America components but when the plants were allowed to grow three weeks after inoculation before being examined, all Bonny Best plants had symptoms of wilt.

When Bonny Best scions were grafted on to Pan America stocks there was no detectable change in the resistant property of the Pan American stocks. Likewise it was found that the Pan American scions were not able to impart any degree of resistance to the Bonny Best stocks and in fact seemed more susceptible to the disease than the Bonny Best scions. May (1930) found that a Norton scion grafted on to a Bonny Best stock developed discolored bundles but that they were not as pronounced in the scion as in the Bonny Best bundles immediately below. The fact that the Bonny Best scions on the Pan America

stocks were still susceptible to the disease when inoculated through the adventitious roots shows that little if any of the resistance of the stock was transmitted to the Bonny Best scion. Bond (1936) grafted several varieties of tomatoes, some resistant and some susceptible to *Cladosporium fulvum* Cke. and found that the stock and scion retained their characteristic reaction to infection without regard to the reaction of the scion or stock grafted to it.

The Pan America scion, although very susceptible to the tomato wilt fungus when inoculated through its Bonny Best stock, showed its resistance again when inoculated through its own adventitious roots. The same degree of resistance was shown by cuttings made from the tops of Pan America plants that had been allowed to root themselves in a sand bench.

Some Solanaceous plants are able to synthesize certain substances in their root systems (Dawson, 1942). Whether the roots of certain varieties or species of tomatoes are able to synthesize materials which prevent the growth or counteract the action of the wilt organism, or whether the resistance is related to some inherited morphological characteristic, remains to be proved. Schmidt (1933) found that the alkaloid solanin had an inhibitory action on certain growth phases of *Cladosporium fulvum* but attributed the resistance of *Solanum racemigerum* (*L. pimpinellifolium*) to a substance of unknown composition termed "prohibitin" that was present in addition to solanin. Some essential nutrient for the growth of the organism may be present in the Bonny Best and absent from the Pan America roots. Brown (1936), in his review, pointed out examples where a low soluble nitrogen content was found to be correlated with resistance to a number of parasites in different plants.

The culture experiments reported in this paper fail to show that any substance inhibitory or toxic to the tomato wilt fungus was extracted from the resistant Pan America plants. However, the active material may have been lost or destroyed during the filtration or autoclaving procedures.

SUMMARY

Approach grafts and splice grafts of the wilt resistant Pan America and the susceptible Bonny Best varieties of tomato plants were made to determine the nature of the resistance in the Pan America tomato plant.

About 40 per cent of the Pan America components of the 142 approach grafts showed some symptoms of disease two or three weeks after being inoculated with *Fusarium oxysporum* f. *lycopersici*. In the majority of cases it was evident that the infection had crossed the graft union.

Bonny Best scions on Pan American stocks remained free of disease in more than 90 per cent of the cases. Pan America scions on Bonny Best stocks became severely infected in nearly all cases as did the Bonny Best scions on Bonny Best stocks. Bonny Best scions have little or no effect on the resistant

properties of the Pan America stock and the Pan America stock does not impart any of its resistant characteristics to the Bonny Best scion. Stocks and scions of *L. pimpinellifolium* plants gave practically the same response as Pan America.

Cultures made of sterile whole tissues and sterile unheated extracts from the tissues of the resistant and susceptible plants failed to indicate the presence of any substance in the resistant plants that was toxic to the tomato wilt fungus.

The outstanding fact observed is the complete susceptibility of the Pan America scions when supported on a susceptible Bonny Best root system. Resistance to the wilt fungus in tomatoes appears to be localized entirely in the root system of the resistant varieties and is not transportable.

U. S. SOUTHEASTERN REGIONAL VEGETABLE BREEDING
LABORATORY,
CHARLESTON, SOUTH CAROLINA

LITERATURE CITED

- BOND, T. E. T. 1936. *Phytophthora infestans* (Mont.) de Bary and *Cladosporium fulvum* Cooke on varieties of tomato and potato and on grafted Solanaceous plants. *Ann. Appl. Biol.* 23: 11-29.
- BROWN, W. 1936. The physiology of host parasite relations. *Bot. Rev.* 2: 236-281.
- DAWSON, R. F. 1942. Nicotine synthesis in excised tobacco roots. *Amer. Jour. Bot.* 29: 813-815.
- LEACH, J. G. 1929. The effect of grafting on resistance and susceptibility of beans to *Colletotrichum lindemuthianum*. *Phytopath.* 19: 875-877.
- MAY, CURTIS. 1930. The effect of grafting on resistance and susceptibility of tomatoes to *Fusarium* wilt. *Phytopath.* 20: 519-521.
- ORTE, W. S., AND H. B. WALKER. 1941. The Pan America tomato, a new red variety highly resistant to *Fusarium* wilt. U. S. Dept. Agric. Circ. 611.
- SCHMIDT, M. 1933. Zur Entwicklungsphysiologie von *Cladosporium fulvum* und über die Widerstandsfähigkeit von *Solanum racemigerum* gegen diesen Parasiten. *Planta* 20: 407-439.
- WELLMAN, F. L. 1939. A technique for studying host resistance and pathogenicity in tomato *Fusarium* wilt. *Phytopath.* 29: 945-956.

CLEISTOGAMY AND CHASMOGAMY IN *BROMUS CARINATUS* HOOK. & ARN.¹

Jack R. Harlan²

CLEISTOGAMY has been reported in numerous grass species, many of which are highly specialized with respect to this type of reproduction. Hackel (1906) divided the grasses into four groups on the basis of their cleistogamous habits, a scheme followed by Uphof (1938). These groups are: (a) the facultatively cleistogamous species, (b) the dimorphic species, (c) the species which are cleistogamous only, and (d) the amphigamous species with the two types of flowers on specialized portions of inflorescences. In the facultatively cleistogamous species the chasmogamous flowers differ little from the cleistogamous flowers in morphology and appearance. In the dimorphic species the two types of flowers are usually quite different and are borne on separate plants. The specialized cleistogamous grass flowers often lack glumes and may be subterranean as in *Amphicarpon*, described by Weatherwax (1934), or hidden in leaf axils. They are sometimes so different morphologically that the plants would be placed in a different tribe were the chasmogamous flowers not available (Chase, 1918).

Bromus carinatus Hook. and Arn. and its relatives are facultatively cleistogamous and do not ex-

hibit extreme specialization in flowers or inflorescences. The first description of cleistogamy in the species was made by Sir William Jackson Hooker (1840) when he described and named the species *Ceratochloa grandiflora*, later reduced to a synonym of *B. carinatus*. He wrote:

"From an examination of more than one spikelet it would appear that the lower florets bear abortive stamens with small short anthers; the upper ones abortive pistils, with very large linear anthers."

While his observations were undoubtedly correct, the interpretation was somewhat at fault since neither pistils nor anthers are aborted, but the marked difference in size of anther in the same spikelet is of frequent occurrence in facultatively cleistogamous flowers.

The nature of the cleistogamy in *B. carinatus* has not been carefully investigated in the 100 years since Hooker named the species. In the course of a related study of variation in this species conducted at the University of California, the author examined and compared the development of the two types of flowers. The results are reported in this paper.

MATERIALS AND METHODS.—Ten strains of *Bromus carinatus*, arbitrarily numbered 1 to 10, were grown in three environments and observations made on the types of inflorescences produced. These environments were: (1) inside the greenhouse with ample water, but inadequate light; (2) outside the greenhouse on an elevated bench with occasional watering; and (3) outside the greenhouse in a cold frame with frequent watering. Plants inside the

¹ Received for publication April 8, 1944.

Contribution from the Department of Genetics, University of California, and completed while the author was employed by the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

² Formerly Research Assistant, University of California, now (1944) Assistant Agronomist, Division of Forage Crops and Diseases.

TABLE 1. *Summary description of ten races of Bromus carinatus used.*

Number	Original locality	Herbarium number	Most distinctive characters
1	Mariposa, Calif. Alt. 2400 feet	Stebbins 2809	Tall, erect; exceptionally long awns and lemmas
2	Berkeley, vacant lots	Stebbins 2745	Medium height; leaves erect; sheaths white, hairy
3	Berkeley, U. C. campus	Tall; panicle fine, nodding; spikelets small, red, many flowered
4	Arlington Ave., Contra Costa Co., 1 mile north of Berkeley	Joranson 4	Medium height; panicle spreading; leaves glabrous
5	Berkeley, Shasta Road	Stebbins 2677	Tall, robust; panicles large; seeds large
6	Mira Vista Country Club, Contra Costa Co.	Joranson 9	Medium height; hairy; frequently sub-prostrate
7	Berkeley, lawn on Cragmont Ave.	Stebbins 2684	Tall; panicle fine; spikelets small, red
8	Oakland, Redwood Road, north of Mills College	Stebbins 2668	Medium height; panicle spreading; spikelets small
9	One-half mile north of Berkeley Country Club, Contra Costa Co.	Joranson 12	Similar to No. 6, but branching more profusely
10	Meadow View Ranger Station, Plumas Co. Alt. 7000 feet.	Short; leaves stiff; awns short

greenhouse were grown somewhat crowded in large flats, while those outside the greenhouse were in separate 6-inch pots. Plants were grown from seeds planted the first of August, some races flowering in the fall and winter, others not flowering until spring. Strain number 1 was originally from a foothill station in the Sierra Nevada Mountains. Strain number 10 was from a high altitude station in the same mountains, while strains 2 to 9 inclusive were originally from Berkeley, California, and environs (table 1).

Race number 6 proved to be particularly suitable for detailed study because of its ability to flower under a variety of conditions. Plants of this race blooming in the fall proved to be cleistogamous, while the same individuals blooming in the spring produced chasmogamous florets. A series of inflorescences from primordial stages to maturity was collected in the fall and preserved until the same plants flowered in the spring. Both cleistogamous and chasmogamous panicles were then carefully dissected and comparable stages compared. Measurements were made of the floral organs, and a series of composite diagrams was prepared, each based upon at least five dissections. Microtome sections were needed in a few cases where the spikelets were particularly small. Variation in the species is considerable, and some strains have florets which when ma-

ture are but one-third the size of those of strain number 6, while others have florets nearly twice as large as number 6. The measurements, then, are for the purpose of comparison between cleistogamous and chasmogamous florets of the same strain.

Numerous observations were also made in the field and more casual examinations made on over 100 races collected from southern Arizona to southern British Columbia.

RESULTS.—*General observations.*—Optimum conditions for flowering usually favored chasmogamy, while adverse conditions tended to force the plant into cleistogamy. Of the ten races growing in the greenhouse, Nos. 5, 6, and 9 flowered openly at least to some extent. Plants of these strains also had cleistogamous panicles. The other strains were entirely cleistogamous in the greenhouse. On the elevated bench, under relatively dry conditions, Nos. 2, 5, 6, 7, 8, and 9, and some plants of No. 3 flowered openly; the others were cleistogamous. In the cold frame with greater moisture, all strains produced some open flowers except number 1 (table 2). This strain, however, when planted in a variety nursery produced open flowers. Of 32 races introduced from Arizona, 23 flowered cleistogamously in the fall when planted in the summer. Most of these same

TABLE 2. *Type of flowering habit shown by 10 races of Bromus carinatus in three different environments.*

Environment	Partly or completely chasmogamous Race number	Exclusively cleistogamous Race number
1. Inside greenhouse with ample water but inadequate light	5, 6, 9	1, 2, 3, 4, 7, 8, 10
2. Elevated bench outside greenhouse with occasional watering	2, 3, 5, 6, 7, 8, 9	1, 4, 10
3. Cold frame outside greenhouse with frequent watering	2, 3, 4, 5, 6, 7, 8, 9, 10	1

racess produced at least some open flowers the following spring.

Similar observations were made in the field. Plants in poor situations such as along paths, in roadways, pastures, etc., are always or nearly always in cleistogamy. Some strains flower on through the summer and into the winter, if there is sufficient moisture. The off-season panicles are always cleistogamous. In fact, except for a comparatively brief period at the height of the flowering season, nearly all the panicles formed are cleistogamous. Evidently the conditions which are most favorable for open flowering in one strain may not be the most favorable for another (table 2). In the field, plants are sometimes observed which appear unable to flower openly even though other plants of another race are doing so nearby. Crowding, shading, poor nutrition, unfavorable day length, unseasonal flowering, or other similar adverse conditions apparently force the flowers to become cleistogamous in varying degrees depending upon the strain.

Development of cleistogamous and chasmogamous panicles.—Cleistogamous and chasmogamous panicles of race No. 6 are compared at comparable stages in their development in the composite diagrams, figures 1 to 3. The measurements refer to measurements of anther length taken on panicles as they flowered in the nursery. Figures 1–3 were drawn to the same scale.

The first composite diagram (fig. 1) shows two panicles, the chasmogamous one not yet having reached the stage of meiosis. The largest spikelet (terminal one) on the cleistogamous panicle has two florets which have undergone meiosis in the anthers. Note the steepness of the developmental gradient of the cleistogamous panicle as evidenced by the fact that the lower portion of the cleistogamous panicle is not completely differentiated. At least two sets of panicle branches are in the primordial stage at the base of the panicle, yet meiosis has already taken place at the upper end. In the chasmogamous panicle none of the florets has undergone meiosis, but the panicle has been completely differentiated for some time.

Figure 2 depicts a later stage where nearly half of the florets of each panicle have completed meiosis. It is to be noted that the lower florets of a spikelet are the first to develop, while the upper spikelets of the panicle are the oldest. In both of these panicles, a number of the upper spikelets have lower florets which have passed meiosis, but the same spikelets have upper florets which have not yet reached that stage. The developmental gradient again is sharper in the cleistogamous spikelets than in the chasmogamous ones. The duration of meiosis appears to be much shorter in the cleistogamous florets, judging by the number of meiotic divisions actually found and by the number of stages present when found. Meiosis in the cleistogamous flowers was found to be normal.

Figure 3 shows two panicles at anthesis. The low-

er florets of the upper spikelets of the chasmogamous panicle are open, as indicated by the florets shown in black, and the anthers and stigmas are exerted. The panicle of the cleistogamous plant appears much younger, but fertilization has actually taken place in a few florets and the cariospores have begun to develop. The anthers are about 0.5 mm. in length at anthesis as compared to more than 6 mm. for the open-flowered panicle. At meiosis, the comparative sizes of the anthers were 0.4 mm. and 3.0 mm. respectively.

Figure 4 shows two spikelets at a stage where the lower florets are undergoing meiosis. The cleistogamous spikelet is drawn from sectioned material since it was too small to dissect well. The chasmogamous spikelet is approximately three times the size of the cleistogamous one, and is not shown in full. Lemmas, paleas, and some of the anthers were removed for clarity. In the cleistogamous spikelets, microsporogenesis and macrosporogenesis take place at very nearly the same time. The macrospores may be formed first, but if so the difference in time is very slight.

Cleistogamous ovaries are sometimes fertilized before the panicle is exerted from the upper sheath. At the stage depicted in figure 2, the cleistogamous panicle was completely enclosed in the upper sheath, while the chasmogamous panicle was partially exerted. In the stage shown in figure 3, the open-flowered panicle had been exerted for a period of about two weeks, while the cleistogamous panicle was just beginning to become exerted.

The ovaries differ somewhat in size, but the discrepancy is not nearly so great as that between the two types of anthers (table 3). The lodicules of the chasmogamous florets are much better developed than those of the cleistogamous florets. In the former case, they are almost as long as the ovary and become very stiff and hard, while in the latter case they are very thin, soft, and difficult to find.

When the seeds are mature, it is possible to determine whether or not they came from long-anthered or short-anthered florets. The short stamens become relatively firmly fixed to the developing ovary, the filament adheres to the ovary and the anthers themselves stick to the stigma hairs. The long anthers are usually lost after anthesis, but are frequently present in sterile florets at maturity. This explains the "abortive" anthers observed by Hooker (1840). It also permits a classification of seed collected in the field.

Actual studies of the fertilization of the cleistogamous florets were not made. That true fertilization takes place seems evident from the following observations: (1) Pollen is shed from the anthers and germinates on the stigma, and (2) segregation was clearly detected in F_2 populations derived from cleistogamously produced seeds of the F_1 in a number of interracial hybrids.

Cleistogamous and chasmogamous florets on the same panicle.—In the course of the flowering sea-

TABLE 3. Comparative sizes of floral organs in cleistogamous and chasmogamous florets at meiosis and anthesis.

Organ	Chasmogamous	Cleistogamous
Meiosis		
Ovary	0.9 mm.	0.4 mm.
Stigma	0.6 mm.	0.3 mm.
Anther	3.0 mm.	0.4 mm.
Anthesis		
Ovary	2.3 mm.	1.8 mm.
Stigma	1.6 mm.	0.5 mm.
Anther	6.5 mm.	0.5 mm.

son, several races produced first cleistogamous panicles then open-flowered ones, and finally cleistogamous ones again. In passing from the cleistogamous flowering habit to the chasmogamous habit or from chasmogamy back to cleistogamy the plant may produce a panicle containing both types of florets. Several panicles of this type were mapped, each spikelet being examined and charted according to the type of floret. The panicle was marked off by nodes which were designated by letter, and the spikelets systematically numbered according to the size of the branch and position on the branch (fig. 5). The transition from short to long anthers proceeds with perfect regularity. The oldest florets in each case are short-anthered ones; the younger florets are long-anthered. The basal florets of a spikelet are older than the upper florets of spikelets immediately above it on the panicle.

In a few cases, two sizes of anthers occurred in a single floret. In such cases the short anthers dehisced long before the long anthers. In some cases, the small anther sheds its pollen about the time the long one is undergoing meiosis. Florets with two short and one long anther were observed as well as those with one short and two long anthers. Apparently the median or dorsal anther differentiates a little before the other two. If the inflorescence is passing from chasmogamy to cleistogamy, the median anther is the long one, and the lateral anthers are short. If the inflorescence is passing from cleistogamy to chasmogamy, as diagrammed here, the median anther is short and the lateral ones long.

Figure 6 shows schematically the transition from long- to short-anthered florets based upon the actual observations made. The florets along the demarcation line are approximately the same age, being differentiated at about the same time. It is most probable that the chasmogamy or cleistogamy of a floret is determined at the time of differentiation in the primordia. The transition would appear to be physiologically abrupt despite the general nature of the factors which induce cleistogamy. Such an abrupt change might be explained on a threshold basis. If conditions become sufficiently favorable, a threshold is passed permitting the plant to produce open flowers. If the conditions of this threshold are not maintained, the plant must pass into cleistogamy.

DISCUSSION.—The above observations on the nature of the cleistogamous flower in *Bromus carinatus* do not deviate fundamentally from reported observations on certain other species. The subject of cleistogamous flowers is reviewed by Uphof (1938). The group in which cleistogamy has been most studied is the Violaceae. A number of species of this family show facultative cleistogamy and numerous parallels can be drawn between the nature and function of these flowers and those of *B. carinatus*. Theron (1939) in describing the cleistogamous flowers of *Viola odorata* remarks that the cleistogamous flowers appear to be morphologically arrested, but are in reality very precocious in their maturation. Uphof (1938) generalized on facultative cleistogamy as follows:

"In all cases it has been evident that the condition of cleistogamy represents a flower-bud which is arrested by some cause and at some point in its normal chasmogamic development."

Perhaps it might be as appropriate to say that the cleistogamous flowers result from a flower bud whose maturation is speeded up at some point in its normal chasmogamous development.

Intermediate flower types have often been observed, not only in the Violaceae, but in a number of unrelated species. The early work of Vöchting (1893) showed that different amounts of light affect the chasmogamy or cleistogamy of the flower and produce intermediates in a number of species. Intermediate types of flowers are reported for *Triodia* (Poulter, 1932; Beddows, 1931); *Linaria* and *Antirrhinum*, Brandegee (1900); *Viola* (Goebel, 1905; Bergdolt, 1932; Brunn, 1931, and others); and for *Commelinantia* (Parks, 1935). Variation in the intensity of the expression of the cleistogamous habit is also reported in *Viola purpurea* by Uphof (1934). The cleistogamous flowers of poorly nourished plants terminated their growth at a much lower stage of development than did those of otherwise normally developed plants. Moreover they appeared much earlier. Cleistogamous flowers of this species could easily be converted to chasmogamous ones when sufficient food was available. Correns (1926, 1930) found a genetical basis for different percentages of cleistogamous and chasmogamous flowers in different races of *Lamium amplexicaule* L. A ratio of 1:2:1 was demonstrable.

While the cleistogamous flowers of *Viola* resemble those of *Bromus* in their precocity, their response to environment and their exhibition of intermediate forms, they differ in certain other respects. In the first place, the pollen of cleistogamous flowers of *Viola* is not shed, but germinates in the anther sacs and the pollen tubes reach the style without being discharged. This habit is reported also in *Lespedeza* by Hanson (1943). A more fundamental difference which affects the breeding structure is the fact that the chasmogamous flowers of these species of *Viola* do not function (Madge, 1929). The genetic effect of this form of reproduction is but one step removed

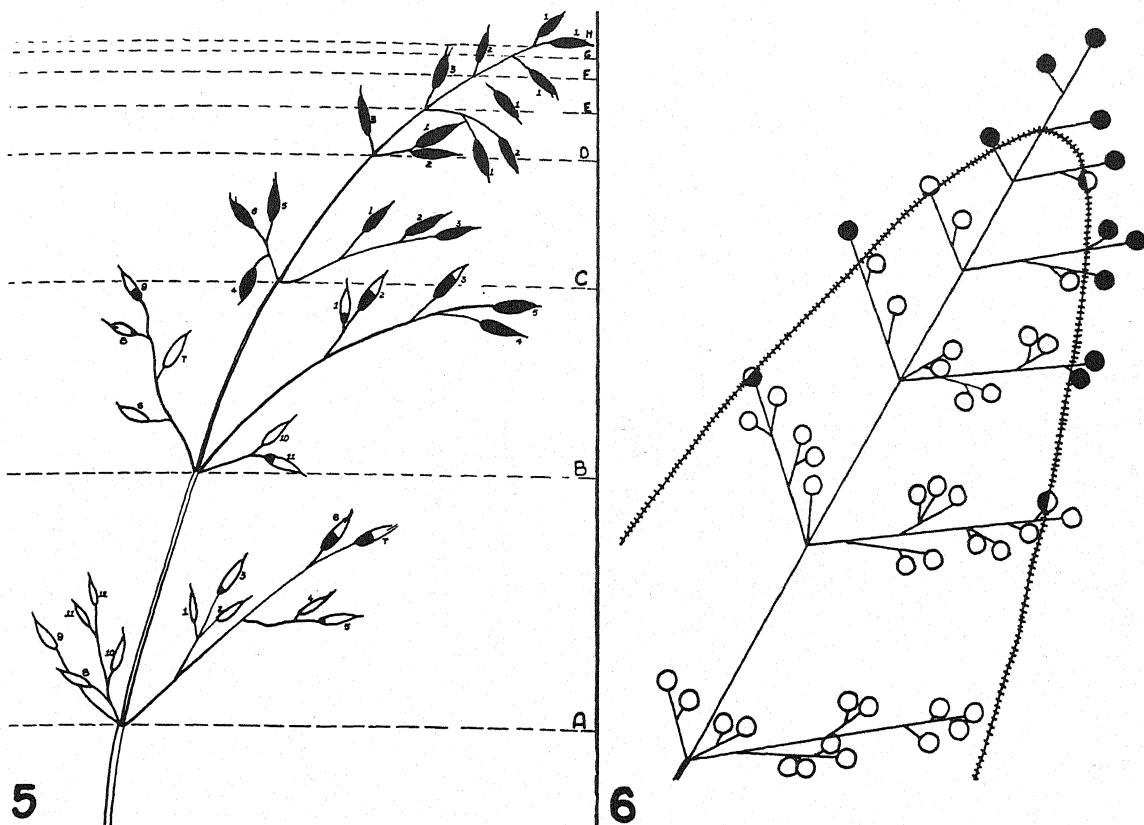


Fig. 5-6.—Fig. 5. Sample map of panicle in which both chasmogamous (light) and cleistogamous (black) florets were found.—Fig. 6. Schematic diagram showing orientation of chasmogamous (light) and cleistogamous florets (black).

from apomixis. Since true fertilization takes place in *Viola*, the plants are very nearly or completely homozygous where cleistogamy is the usual mode of reproduction. In this respect they differ from apomictic plants, but because the chasmogamous flowers do not function a complete interplant isolation exists. The races which result from this isolation are closed systems from the evolutionary point of view and cannot be modified except by mutation. In *Bromus carinatus*, however, cross pollination may occur in the chasmogamous flowers and interplant isolation is only partial.

SUMMARY

Observations were made on the appearance of cleistogamous florets in plants of *Bromus carinatus* grown under different environmental conditions. Comparisons were made at several stages of development between cleistogamous and chasmogamous panicles.

Bromus carinatus is facultatively cleistogamous, producing both chasmogamous and cleistogamous florets on the same plant.

Optimum conditions for flowering induce chasmogamy; adverse conditions induce cleistogamy.

The developmental gradient in cleistogamous spikelets and inflorescences is much steeper than the

gradient in the chasmogamous spikelets and inflorescences.

Both types of florets may appear on the same panicle, due to an abrupt change in the developmental gradients at an early stage in the development of a panicle.

This steepness of developmental gradient in cleistogamous spikelets is associated with marked precocity in maturation of both ovary and pollen.

Morphologically, cleistogamous flowers differ from chasmogamous ones in having smaller floral organs, particularly anthers, stigmas, and lodicules.

SOUTHERN GREAT PLAINS FIELD STATION,
WOODWARD, OKLAHOMA

LITERATURE CITED

- BEDDOWS, A. R. 1931. *Triodia decumbens* Beauv. Ann. Bot. 45: 443-451.
- BERGDOLT, ERNST. 1932. Morphologische und physiologische Untersuchungen über *Viola*. Zugleich ein Beitrag zur Lösung des Problems der Kleistogamie. Botanische Abhandlungen, herausg. von K. Goebel. Heft 20: 1-20. Jena.
- BRANDEGEE, T. S. 1900. Cleistogamic flowers in *Scrophulariaceae*. Zoe 5: 3.
- BRUNN, H. G. 1931. A theory of the cytologically irregular species *Viola canina* L. Hereditas 16: 63-72.

- CHASE, AGNES. 1918. Axillary cleistogenes in some American grasses. *Amer. Jour. Bot.* 5: 254-258.
- CORRENS, C. 1926. Genetische Untersuchungen an *Lamium amplexicaule* L. I. *Biol. Zentralbl.* 46: 67-79.
- . 1930. Genetische Untersuchungen an *Lamium amplexicaule* L. IV. *Biol. Zentralbl.* 50: 7-19.
- GOEBEL, K. 1905. Chasmogamie und Kleistogamie bei *Viola*. *Allg. Bot. Zeit.* 95: 234-239.
- HACKEL, E. 1906. Über Kleistogamie bei Gräsern. *Oster. Bot. Zeit.* 56: 81-88, 143-154, 180-186.
- HANSON, C. H. 1943. Cleistogamy and the development of the embryo sac in *Lespedeza stipulacea*. *Jour. Agric. Res.* 67: 265-272.
- HOOKE, W. J. 1840. *Flora Boreali-Americana*. London.
- MADGE, MARGARET A. P. 1929. Spermatogenesis and fertilization in the cleistogamous flower of *Viola odorata*, var. *praecox* Gregory. *Ann. Bot.* 43: 545-577.
- PARKS, MABEL. 1935. Embryo sac development and cleistogamy in *Commelinantia pringlei*. *Bull. Torrey Bot. Club* 62: 91-104.
- POULTER, ANNIE A. 1932. Occurrence of "cleistogenes" in certain grasses. *Nature* 129: 690-691.
- THERON, A. 1939. Recherches morphologiques et cytologiques sur les fleurs de *Viola odorata*. *Rev. Cytol. et Cytophysiol. Veg.* 4: 101-118.
- UPHOF, J. C. TH. 1934. Vergleichende blütenmorphologische und blütenbiologische Studien an *Commelina virginica* L. *Ber. Deutsch. Bot. Ges.* 52: 173-180.
- . 1938. Cleistogamic flowers. *Bot. Rev.* 4: 21-49.
- VÖCHTING, HERMANN. 1893. Über den Einfluss des Lichtes auf die Gestaltung und Anlage der Blüte. *Jahrb. Wiss. Bot.* 25: 186-187.
- WEATHERWAX, PAUL. 1934. Flowering and seed production in *Amphicarpon floridanum*. *Bull. Torrey Bot. Club* 61: 211-215.

PERICLINAL CHIMERAS IN DATURA IN RELATION TO THE DEVELOPMENT AND STRUCTURE OF THE OVULE¹

Sophie Satina

THE USE of periclinal chimeras in ontogenetic studies of floral organs has been shown in a series of recent publications on *Datura* (Satina and Blakeslee, 1941, 1943; Satina, 1944). Various types of periclinal chimeras were obtained from seeds treated with colchicine (Satina, Blakeslee and Avery, 1940). This drug may increase the number of chromosomes in affected cells giving rise to $4n$ and $8n$ tissues. In periclinal chimeras which result from colchicine treatment only a single one of the three layers in the primary shoot apex may be polyploid, and hence distinguishable from the others. It has been possible to trace the contribution of the outermost layer (L I), the second layer (L II) and the innermost layer (L III) to the development of the various floral organs, by labelling such layers by means of differences in chromosome numbers.

Various types of periclinal chimeras have been used again in the present paper, the subject of which is the initiation and earliest development of the ovule and the growth and structure of the ovular coat.

The importance of understanding the structure and function of the ovule in every detail is evident. Full and correct information about the body whose primary function is to bear the female gamete and to become the seed will lead to a better understanding of its origin and development and of the processes directly connected with the mechanism of nutrition and growth of the embryo sac. It also might throw some light on a number of problems connected with plant physiology and plant breeding, such as seed dormancy, seed failure in incompatible crosses, etc.

¹ Received for publication October 30, 1944.

Contribution from the Department of Botany, Smith College, New Series No. 15. This investigation was supported in part by the Carnegie Institution of Washington.

The author wishes to thank Dr. A. F. Blakeslee for his help and valuable suggestions throughout this work.

Much is known about the structure and function of the megaspore from numerous studies which have been made on the morphology and cytology of the megaspore mother cell and the embryo sac before and after fertilization. Much less attention has been paid so far to the remaining constituents of the ovule, the cells forming the integument, and the cells of the nucellus, particularly those cells at the chalazal end of the ovule. Studies on the structure, function and fate of these ovular tissues are incomplete. Usually only brief statements about the nucellus and integument accompany the detailed studies on the megaspore; the chalaza, namely, the cells located between the embryo sac and the funicle in a developing ovule, is generally neglected. In some respects the conclusions regarding the fate of the nucellar cells and of the integument seem to be contradictory, probably because of a great variability in the structure and development of ovular tissues in plants belonging to a wide range of species. There are also apparently misinterpretations, the most frequent of which are connected with the nature of the layer adjacent to the embryo sac. Dahlgreen (1927) lists a number of papers in which the innermost layer of the integument is considered a part of the nucellus. It seems that the same misinterpretations are found in other recent papers (Cooper and Brink, 1940; Cook, 1924). Such misinterpretations are apparently the result of a lack of a proper definition of the nucellus, a fact also recognized by Dahlgreen (1927). The definitions found in the literature are rather vague and inconsistent. In speaking of the young ovule the nucellus in current literature is described as the inner cellular mass of the ovule, or as the central parenchymatous mass of the ovule. In the mature ovule, however, the generally accepted definition considers the nucellus as only that part of the ovule which is surrounded by the integument, and the integument is described as arising from or

at the base of the nucellus (Bower, 1919; Engler and Prantl, 1926; Strasburger, 1930; Goebel, 1933; Haupt, 1934; Pearson, 1932-33; Wettstein, 1935), or it is even stated that the integument is grown "congenitally" with the funiculus (Dahlgreen, 1928). Should the nucellus be interpreted only as that portion of the ovule which is covered with the integument? Should such an interpretation be accepted in the case of species where the integument starts to develop near the apical portion of the nucellus and not at or from its base? In *Datura*, as in *Lycopersicon* (Cooper, 1931), in *Solanum* (Rees-Leonard, 1935) and in *Ulmus* (Walker, 1938) the integument arises as an outgrowth of the epidermis at the level just below the archesporial cell, and continues its growth outward around the upper portion of the nucellus. There is a large mass of parenchymatous nucellar tissue below the archesporial cell, which is not invested by the integument. It would seem more consistent to consider this tissue as nucellar in both young and mature ovules. As will be shown in later paragraphs these cells take an active and important part in nourishing and protecting the embryo sac and in the formation of the seed coat. Moreover, they are of the same origin as those nucellar cells which are surrounded by the integument. The present paper, therefore, will use the term nucellus to include not only that portion of the ovule invested by the integument, but also the ovular tissue between the megaspore and the funicle.

The earlier papers on the nucellus and the integument will not be referred to here. The literature concerning the nucellus is covered by Dahlgreen (1927) in his review of the morphology of the nucellus, and by Schnarf (1929). A list of papers on the integument can be found in the comprehensive and detailed work by Souèges (1907) relating to the structure and development of the seed coat in over 140 species of the Solanaceae, including *Datura stramonium* and four other *Datura* species. Souèges summarized the scattered and incomplete observations on the various changes which take place in the ovular layers during the development of the ovule. He added a number of important details on the formation of the single integument. Our present knowledge of the structure and function of the tissues which form the seed coat in *Datura* and other Solanaceae is primarily based on his work. According to Souèges, when the embryo sac is ready for fertilization, three layers can be distinguished in the ovular coat of the Solanaceae: (a) an outermost layer (b) a median layer and (c) an innermost layer. Both the outermost and innermost layers develop from the epidermis and each is one cell thick. The median layer which has an inner and outer zone, each several cells thick, develops from cells which lie within the epidermis. Only the outermost layer of the integument has a purely protective function. The function of the median and of the innermost layers can be considered protective only at early stages. The function of the median layer is primarily nutritive; the func-

tion of the innermost layer is primarily digestive. This statement is in accord with Guignard (1893) who studied the structure of the seed coat in other plants. In recent papers on the Solanaceae, Svensson (1926) also attributes a digestive function to this layer, while Young (1923), Cooper (1931) and Rees-Leonard (1935) consider it to be nutritive. Several names have been used in the literature to designate this innermost layer of the integument in various species. In the present paper it will be referred to as the endothelium.

According to Souèges, during the growth of the ovule a number of cells have digestive properties and the digestive function passes from one cell or group of cells to another at various stages of development of the ovule; from the m.m.c.² to antipodal cells, from these to the endothelium and then to the endosperm.

The structure of the embryo sac when ready for fertilization was described for *Datura stramonium* (*D. laevis*) by Guignard (1902). It has the common eight nucleated structure. Details on megasporogenesis and on the formation of the embryo sac in the same species were given by Satina and Blakeslee (1935, 1937) and need not be repeated here. The brief statements made on this subject by Glišić for *D. metel* (1928) are in agreement with these observations on *D. stramonium*. There is also agreement in respect to the development of the endosperm in both species. The planes of the first two divisions in *D. stramonium* follow the order described by Glišić in *D. metel*, i.e., the first division is transverse and the second is longitudinal. It is not possible to agree with Guignard (1902), who claimed that in *D. laevis* (*D. stramonium*) the first three divisions in the endosperm are transverse. Both *Datura* species thus belong to the same group and *D. metel* should not be considered intermediate between *D. stramonium* and *Hyoscyamus*, as has been assumed by Glišić (1928), who based his conclusion on Guignard's statement.

It has been shown in a recent paper on *Datura* that in the young ovaries ready for the formation of ovules, the placenta is built up primarily by the cells of the innermost layer (L III) and that at this stage the epidermal layer (L I) and the subepidermal layer (L II) each supply only one layer of cells (Satina and Blakeslee, 1943). A few additional L II cells are sometimes formed apparently because of premature periclinal divisions in the subepidermal layer of the placenta (fig. 1). However, as can be seen in figures 1, 2, 11 and 12, it is the L III layer in the placenta which actually initiates the formation of ovules. Groups of L III cells under the subepidermal layer begin to multiply rapidly, forming the bulk of numerous projections. Each projection is covered with one layer of L II cells and one layer of epidermis. These young ovules are scattered all over the previously smooth surface of the placenta, which becomes undulate. The initiation of the ovules begins in the upper portion of the placenta and con-

² m.m.c. = megaspore mother cell.

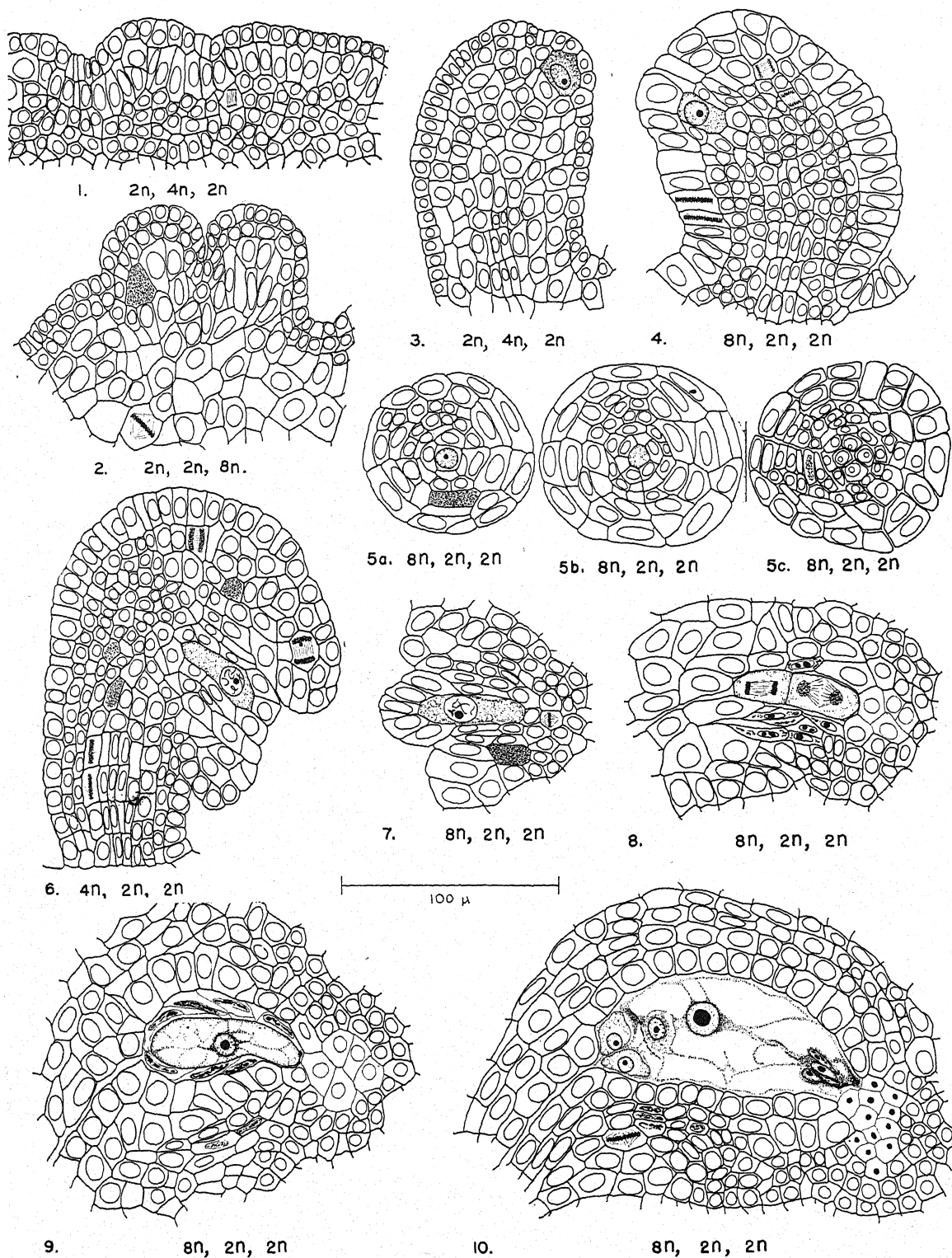


Fig. 1-10. Periclinal chimeras, ovule development.—Fig. 1-4, 6-10, long. sections, fig. 5a-5c, transections.—Fig. 1. Placenta, initiation of ovules by the $2n$, L III layer.—Fig. 2. Same, initiation by the $8n$, L III layer.—Fig. 3, 4. Young ovules: nucellus with an archesporial cell, provascular cells. Early development of the integument from epidermal cells.—Fig. 5a-5c. Ovule, sections cut at different levels: (a) near the top of the m.m.c.; (b) through the median portion of the m.m.c., and (c) below its base. The number of $2n$ nucellar cells increases toward the base. The $8n$ integument is more developed on the upper side of the ovule (right) than on its inner side (left).—Fig. 6. Same stage. Nu-

tinues downward. Each ovule is separated from the adjacent ovules by the epidermal and subepidermal cells of the placenta. These cells also divide intensively thereby adding to the surface of the ovule, which rapidly increases in volume, and to the tissue separating the adjacent ovules (fig. 2). The earliest stages of ovule development are shown in figure 1. In this longitudinal section of the placenta of a $2n$, $4n$, $2n$ chimera the two projections seen at the left are formed predominately by diploid L III cells. The single L II layer is tetraploid. The initiation of two more ovules is seen on the right where division in the two groups of L III cells began later and where the surface of the placenta is still almost smooth. The initiation of these two ovules can be recognized, however, by the additional L III cells which form an angular line of diploid cells under the tetraploid subepidermal layer. The initiation of two more ovules is seen in another $2n$, $4n$, $2n$ chimera (fig. 11). The beginning of ovule formation in $2n$, $2n$, $8n$ chimeras is shown in figures 2 and 12. As in the earlier stage just discussed, protuberances are formed predominantly by L III cells. The L III layer in these chimeras is octoploid and can be easily distinguished by the size of its cells from the diploid subepidermal L II layer. The section through the ovule on the very right in figure 12 is not median and the diploid cells seen in this ovule belong to the subepidermal and epidermal layers covering the inner portion of the ovule.

The ovules seen in figure 11 are about $24\ \mu$ thick; those in figures 2 and 12 are about $48\ \mu$ thick. Very soon after initiation, the divisions of L III cells in the young ovules become less frequent. Instead, a rapid multiplication of cells begins in the subepidermal layer L II, so that for a certain period the further growth of the ovules depends primarily on the contribution of the L II layer. The cells divide in various planes and form the nucellus, from which the sporogenous tissue will differentiate. In later stages, cells from L III are seen only at the base of the ovule (fig. 3, 4), and still later in the funiculus, where they form the single vascular bundle (fig. 6, 16, 17, 19, 23). When the ovule becomes about $70\ \mu$ thick (fig. 3, 4) the archesporial cell is already easily distinguished from the rest of the cells of the nucellus. It is larger and stains differently. The archesporial cell differentiates from the subepidermal layer of the nucellus near the apex of the ovule (fig. 3, 4, 14). It is not rare, however, to find ovules with two, three or more archesporial cells (fig. 13). They are either all subepidermal, lying side by side, or some may differentiate from the layer below. Such is the case in figure 13, but the fourth archesporial cell suggested in this figure is clearly evident in the next section. Usually only one cell develops into the

m.m.c. and goes through reduction division, growth in the other archesporial cells being arrested. However, a few cases have been observed in *Datura* where at least three archesporial cells have become initiated in the subepidermal and the hyposubepidermal layers and have gone through reduction division. Twin embryos have been reported as not infrequent in *Datura* (Satina, Blakeslee and Avery, 1934). It is clear that potential archesporial cells are located not only in the subepidermal layer of the nucellus but also below it. This is in agreement with the observations made in some other species of the Solanaceae (Rees-Leonard, 1935; Lesley, 1926). More information on this subject in other species is given by Dahlgreen (1927) and by Schnarf (1929).

Mature ovules in the Solanaceae are usually classified as anatropous or slightly campylotropous (Souèges, 1907). Applying Goebel's classification (1933, p. 2003) the ovules of *Datura* must be considered anatropous only for a short period of their development. When the megaspore is forming they are rather campylotropous (fig. 18, 21–23) or intermediate between anatropous and campylotropous (fig. 24). After fertilization they become slightly amphitropous. Such changes in position of the ovule during development are not rare in plants according to Goebel (1933, p. 2004). There is a great variation of types of ovules in *Datura stramonium*. Their position depends apparently on their location on the placenta and on their number. In rare cases some ovules were found to be quite erect or atropous in late stages of development, but as a rule in this species the ovules are erect only in the very early stages. Their inversion begins before the m.m.c. divides meiotically and is connected with the development of the integument.

When the archesporial cell begins to differentiate from the nucellus it is covered at the top by a single epidermal layer. Soon some epidermal cells lying on both sides of the archesporial cells divide periclinally. This happens when the ovule is still almost erect. It is the beginning of the formation of the single integument of the ovule. The first additional epidermal cells resulting from periclinal divisions in *Datura* are formed at the level of the base of the archesporial cell, but not at the base of the nucellus as is usually stated in current literature. This early development of integument is seen in ovules of two $8n$, $2n$, $2n$ chimeras, where the epidermis is octoploid (fig. 4, 15) and in two ovules of $2n$, $4n$, $2n$ chimeras, where it is diploid (fig. 3, 14). The ovule curves at the region where the integument begins to develop and the inversion occurs as a result of more intensive growth on one side of the ovule. As is seen in figures 4, 6, 14, 17, fewer cells have divided in each ovule on the inner side of the curving body.

merous $2n$ nucellar cells behind the m.m.c.—Fig. 7. Prophase in m.m.c., nucellar cells on its sides still appear normal.—Fig. 8. Second anaphase in m.m.c.; few disorganized $2n$ nucellar cells at the base and on the sides of the m.m.c.—Fig. 9. Same, later stage. Cells of $8n$ innermost layer of integument are ready to replace the dead nucellar cells on the sides of the megaspore.—Fig. 10. Embryo sac, endothelium with an open space at chalazal end, disorganized $2n$ nucellar cells outside the endothelium.

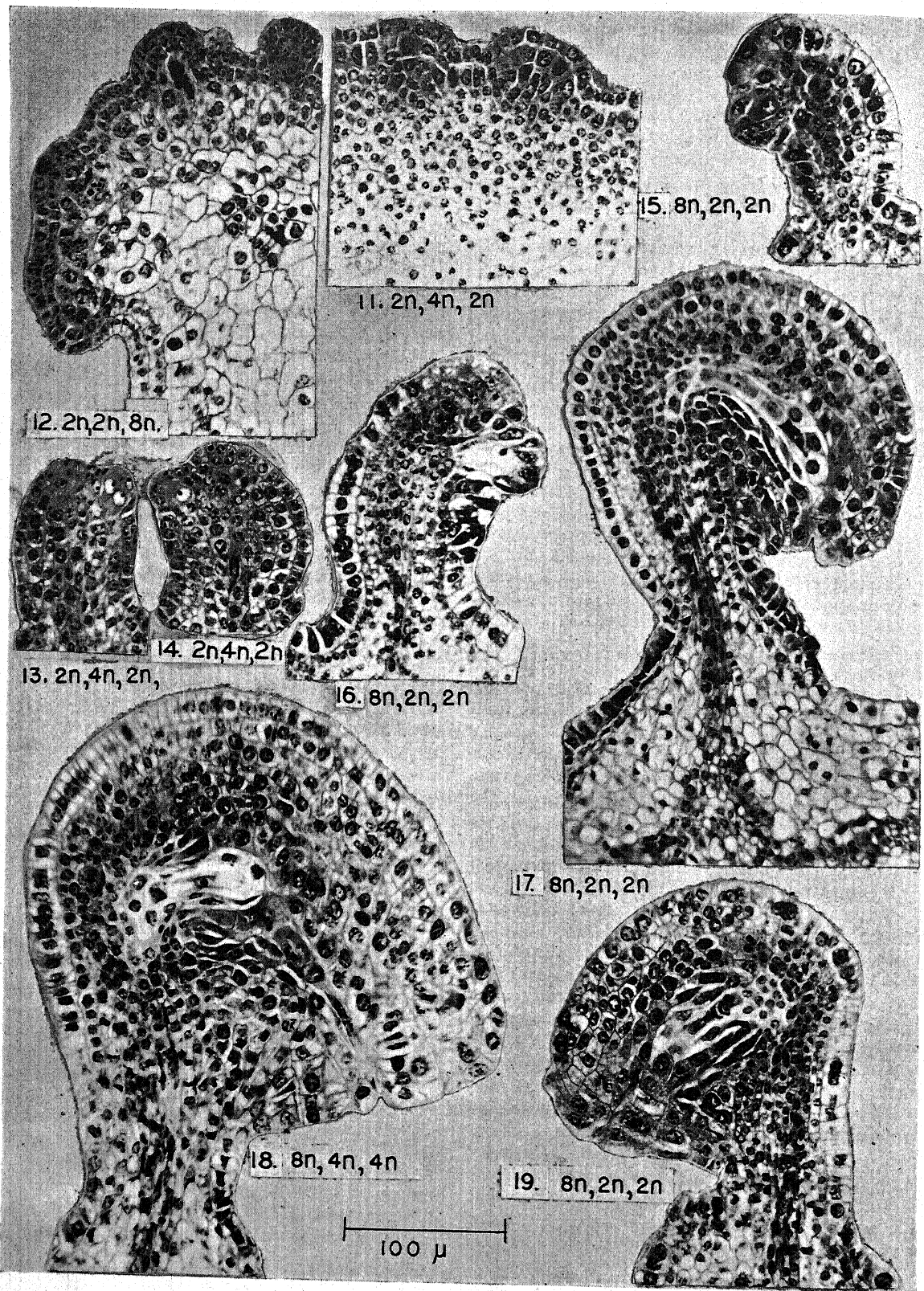


Fig. 11-19. Periclinal chimeras. Ovule development. Photomicrographs. Long. sections.—Fig. 11. Placenta, initiation of ovules by the $2n$, L III layer.—Fig. 12. Same by the $8n$, L III layer.—Fig. 13. Nucellus with three archesporial cells.—Fig. 14-16. Young ovules, single archesporial cell in each. Early development of integument from epidermal cells at

From early stages, the cells of the outer side multiply more rapidly than the cells on the inner side. This explains why the integument has a larger number of cell layers on the outer or convex side of the ovular coat, than on the inner or concave side, in the early as well as in the mature stages (fig. 16, 17, 20, 21). The inversion is accompanied by an elongation of the nucellar cells around the m.m.c. and of the m.m.c. itself, and by multiplication of the cells which form the integument. This results in a continuous outward growth of the distal region of the ovule. In later stages the micropylar end of the ovule turns toward the funicle (fig. 17, 20, 21). The integument at its base is united to the cells of the nucellus near the chalazal end. The part of the integument which develops on the inner side of the inverted ovule becomes adherent to the funiculus (fig. 20–22, 24).

At early stages, all the cells adjacent to the m.m.c. are nucellar with the exception of those of the epidermis at the top of the ovule. In figures 3 and 14 the nucellus is tetraploid; in figures 4 and 15 it is diploid. After the differentiation of the archesporial cell, the cells in the L II layer, which contributed to the formation of the nucellus, begin to divide less frequently. In later stages, when the m.m.c. is ready to divide meiotically the cells of the nucellus around the m.m.c. stop dividing and the multiplication of the nucellar cells continues only in the lower portion of the nucellus—in the chalaza (fig. 6, 7). Until after fertilization, these dividing nucellar cells contribute chiefly to the width and thickness and less to the length of the ovule. The growth in length of the latter from now on until the embryo sac is formed depends primarily on the increase in size of the megaspore and on the multiplication of cells of the L I layer. The L I layer alone forms the integument in *Datura*. The integument rapidly develops in length, width and thickness and soon overgrows the m.m.c. Except at the base where the integument never develops it surrounds this cell almost entirely leaving a very narrow opening—the micropyle—at the distal end (fig. 18). The beginning of the formation of the micropyle is seen in figure 17 at the stage when the m.m.c. has just divided. It will be a long time before the megaspore cell will divide mitotically. The cell increases considerably in length and width and becomes about three times as long as wide before it is ready for the three successive divisions resulting in the formation of the 8-nucleate embryo sac. Together with the growth of the megaspore a marked increase in size is seen in the integument.

In early stages the cells of the integument are adjacent to the m.m.c. only near its top (fig. 6, 7, 15). The median and basal portions of the m.m.c. are surrounded by nucellar cells. In older stages, when the megaspore has formed, the integument replaces the nucellar cells along the sides of the megaspore and becomes adjacent to it all along its length

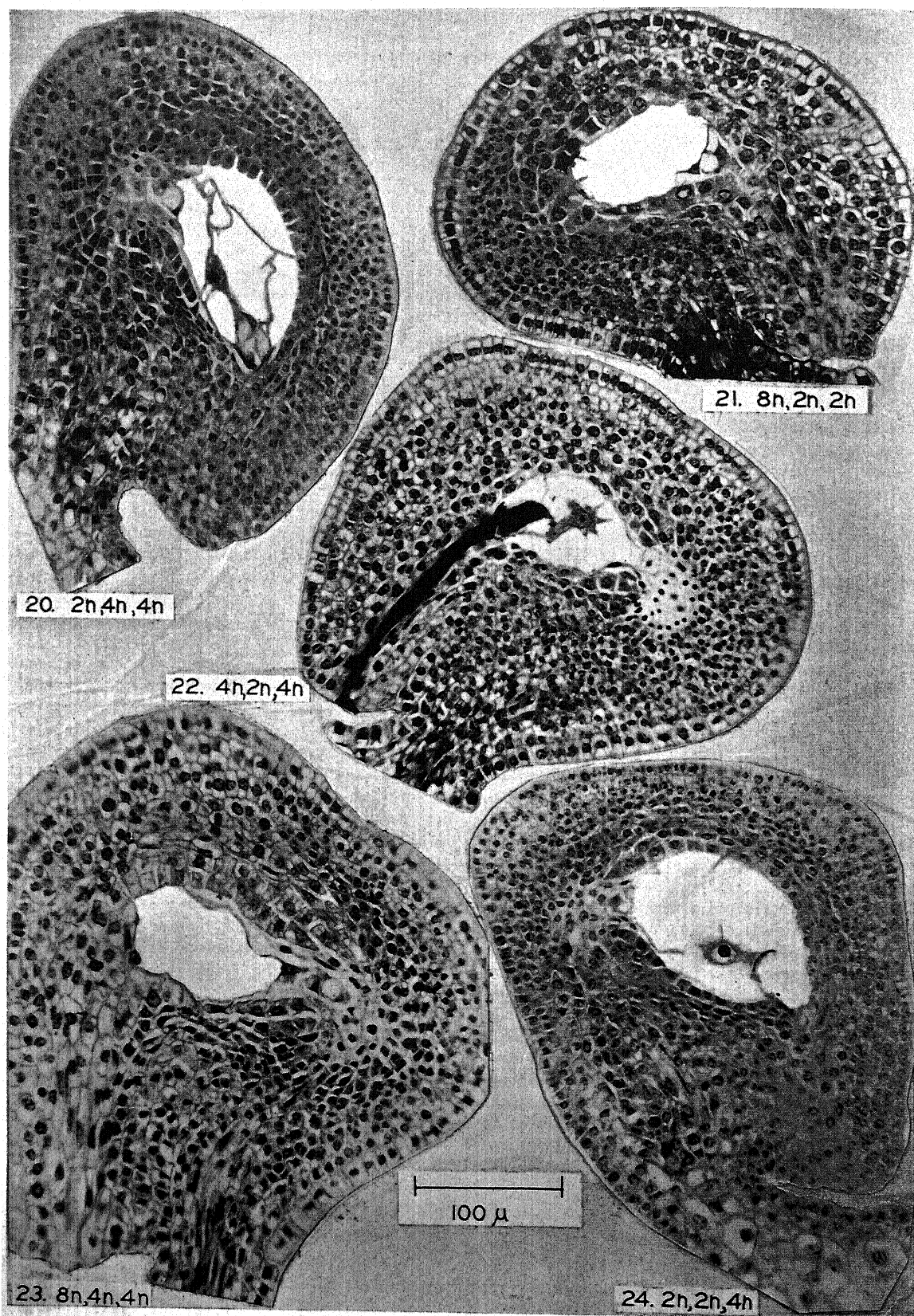
(fig. 8, 9, 16, 17). This happens because there is a continuous disorganization of the nucellar cells which lie around the megaspore. The innermost cells of the nucellus gradually become digested and absorbed by the growing m.m.c. (fig. 7, 8, 16, 17). The outer nucellar cells which successively become adjacent to the megaspore when they replace the already digested cells are also soon disorganized (fig. 9, 18). When all the nucellar cells along the sides of the megaspore have been absorbed by the latter, they are replaced by the cells of the innermost layer of the integument. The last cells of the diploid nucellus which are considerably disorganized, are seen in figure 9. This longitudinal section was made through an ovule of an $8n, 2n, 2n$ chimera. The formation of the endothelium which lines the megaspore is almost completed. The cells in this layer differ from other cells in shape and by the neat order they assume. They are elongated perpendicularly to the long axis of the megaspore and form an almost complete circle around it. It is only at the base of the megaspore that the nucellar cells remain adjacent to it.

The relative number of cells of the integument in comparison with those of the nucellus around the m.m.c. can best be studied in transverse sections of ovules cut at different levels. Three sections cut respectively near the top of the m.m.c. (fig. 5a), through its median portion (fig. 5b), and slightly below its base (fig. 5c), were made through a young ovule of an $8n, 2n, 2n$ chimera. The m.m.c. in this ovule had not yet divided meiotically. In figure 5a the number of octoploid integument cells predominates over that of the diploid nucellar cells. There are more diploid nucellar cells around the m.m.c. in the median level of the ovule (fig. 5b), but the proportion between the number of integument cells and of nucellar cells remains approximately the same. In figure 5c the cells of the nucellus are more abundant than those of the integument. Four diploid cells with larger nuclei in the center of the section are also nucellar. They will be discussed later. Transverse sections through older ovules invariably showed a decrease in the number of the nucellus cells and an increase in the number of cells forming the integument.

At the stage when the embryo sac is ready for fertilization, it is almost completely surrounded by the endothelium which has come from L I. Only a narrow space about 24μ in thickness, is left free from the endothelium near the base of the antipodal cells at the chalazal end (fig. 10). This space remains open and serves as a contact, before and after fertilization, between the embryo sac and the basal portion of the nucellus (fig. 18, 20–24).

Distinct changes also take place, during the development of the ovule, in a small group of nucellar cells located at the proximal end of the m.m.c. and the embryo sac. This group may sometimes be dis-

the top of the ovule.—Fig. 17–19. Later stages. Formation of the micropyle, chalazal pocket, and funicle with a vascular bundle. Disorganization of $2n$ nucellar cells at the sides of the megaspore. Nucellar cells around chalazal pocket appear normal.



tinguished by their larger size in very early stages, soon after the differentiation of the archesporial cell (fig. 4, 5c). It is possible that the early differentiation of such cells indicates a tendency for them to become the additional archesporial cells discussed above. In older stages, when the m.m.c. divide meiotically, a group of nucellar cells at the chalazal end can always be recognized from other cells by larger size, smaller amount of cytoplasm and thinner cell walls (fig. 8, 9). This group of cells becomes completely disorganized when the embryo sac is ready for fertilization. The chalazal pocket replaces them (figs. 20, 22), but it is evident that the disintegration of this group of cells is in progress long before the antipodal cells are formed (figs. 17, 18, 19). The cell contents are digested and absorbed by the m.m.c. and later by the megaspore and not necessarily by the antipodal cells, as has been stated by Souèges (1907). Only vestiges of these nucellar cells are left when the antipodal cells are formed. The size of the chalazal pocket varies considerably and in a few exceptional cases its diameter was found almost equal to that of the mature embryo sac.

Figures 20-24 will help in understanding the origin and structure of tissues in the ovule with the mature embryo sac ready for fertilization. As can be best seen in figure 21, the cells of the octoploid integument and the cells of the diploid nucellus both participate in the formation of the ovular coat which after fertilization will eventually become the seed coat. The integument and the nucellus lie side by side and there is no clearly defined line between them; their cells are intermingled and the proportion of each tissue varies slightly in different ovules. It is evident, however, that at this stage of development the major portion of the ovular coat is contributed by the integument; the micropylar end, and about two-thirds of the outer side of the ovular coat, which is about ten layers thick, are formed by the integument alone. The integument contributes only two or three layers of cells on the inner side of the ovular coat. The chalazal end of the ovular coat and several layers of cells on the inner side of the latter are formed by cells contributed by the nucellus. The base of the embryo sac is embedded in the nucellus and few nucellar cells lying on the sides of the chalazal pocket run at a slightly higher level over its base into the cells of the integument (figs. 20-23). Beyond the chalazal pocket the nucellar tissue is connected with the vascular bundle and the funicle (figs. 23, 24). The contact between the funicle and the micropylar tissue is seen best in figures 20, 21 and 23 because of the difference in cell sizes of the diploid and polyploid tissues forming the funicle and the integument.

In the control plant and in chimeras in which the

L I and L II layers have the same chromosomal constitution, it is hardly possible to distinguish which portion of the seed coat has been contributed by the nucellus and which by the integument. In figure 24, which represents a section of an ovule of a $2n$, $2n$, $4n$ chimera, the nucellus and the integument are both diploid. The larger tetraploid cells seen in the funicle were developed from the L III layer. With the exception of the cells of the epidermis and of the endothelium which are slightly larger and differ somewhat in shape from the rest, there is no distinct difference between cells belonging to the nucellus and those of the integument. The stronger affinity for stain in some layers (Souèges' inner zone of the median layer) is seen in cells contributed both by the nucellus and by the integument (figs. 20, 24), though as a rule the cells at the chalazal end stain darker than those at the micropylar end (figs. 20-24).

The three layers forming the seed coat, as described by Souèges, can be seen and studied better in free hand sections treated by iodine and other reagents than in fixed and stained material. As has been mentioned above, the endothelium and the outermost epidermal cells are usually larger than the cells of the median layer. They also differ in shape (figs. 20-24). About five layers of cells filled with starch and dense cytoplasm represent the inner zone of Souèges' median layer. These cells stain darkly, particularly on the convex side of the ovule. The outer zone of Souèges' median layer is wider than the inner zone and stains lightly, though the cells in this zone also have a considerable amount of reserve food. The cells in this zone continue to multiply after fertilization and add considerably to the growth of the seed coat, while the cells of the inner zone are digested and absorbed by the endothelium. As a result of such disorganization the endothelium becomes almost separated from the rest of the integument. About ten to twelve days after fertilization the endothelium itself is digested and absorbed by the endosperm. The latter digests also the major part of the outer zone of Souèges' median layer. Further details regarding the mature seed coat and the embryo development will be given in a later paper.

DISCUSSION.—The use of periclinal chimeras has been of great help in studying the development and structure of the ovule in early and mature stages. The material collected from chimeras with polyploid epidermis (L I) and diploid median layer (L II) and from chimeras with the reverse combination made it possible to learn in detail the origin and fate of various tissues participating in the formation of the ovule and its coat.

The ovules develop in a different manner from the

Fig. 20-24. Periclinal chimeras, ovules, photomicrographs. Long. sections. The ovular coat has three layers: the epidermis, the median layer and the endothelium. With the exception of an open space, seen at the chalazal end, the endothelium forms an almost complete circle around the embryo sac. At the chalazal end the median layer of the coat is formed by nucellar cells, at the micropylar end and on the sides of the embryo sac it is formed by the integument. The outer side of the integument is wider than the inner.

other organs. There is a distinct alternation in the activity of the cells contributing to the growth of the ovules. As a result of such successive activity of the three layers the ovule can be roughly divided along its length into three portions, one following the other. In each, the funicle, the nucellus, and the integument, the cells derived from one of the three layers, L III, L II and L I, predominate in number over the others.

Cells of epidermal origin alone participate in the formation of the integument. Contrary to Souèges the cells from the inner tissues do not take part in the formation of the integument. Souèges' statements concerning the protective, nutritive and digestive properties of the integument are confirmed by this study. The same is true in regard to his observations on the digestive properties of different cells at various stages: the megaspore, the endothelium and the endosperm. There is no doubt that the development of the embryo sac and its growth in length, width and thickness is directly connected with the disorganization and digestion of the cells lying around the embryo sac. The absorption of these cells serves a double purpose: it supplies the megaspore with nutritive substances and it makes space for the enlarging embryo sac. In accord with Souèges it also has been found that the ovular coat, in ovules ready for fertilization, has three layers, the tissues of which differ in structure and function. There is, however, a great difference in the interpretation of the origin of the ovular coat. According to Souèges (1907) and to Schnarf (1929, 1931), all the nucellus in the Solanaceae is digested long before the embryo sac becomes ready for fertilization. This is true in *Datura* only in respect to the nucellar cells lying around the megaspore and the few cells located immediately beyond its chalazal end. A considerable number of nucellar cells at the base of the integument and around the chalazal pocket remain active and participate, together with the integument, in the formation of the ovular coat. The same is true in *Phaseolus* (Brown, 1917) where the nucellar cells at the chalazal end persist until late stages. They become larger and are arranged in definite rows, which diverge from the point of origin of the integument. The nucellar cells function like Souèges' median layer. They store food and their function before fertilization is predominantly nutritive. Later, after fertilization, they begin to multiply more intensively than the cells of the micropylar end of the seed coat and add much to the growth of the seed. It is the portion at the chalazal end of the seed coat which is predominantly responsible for the size and shape of the mature seed.

There are evidences which indicate that inhibitors which cause seed dormancy in *Datura* are located in the seed coat. They are apparently present in the dead cells which fill the space of the concave surface of the seed. Details regarding this important factor will be given in a later paper. It is mentioned here only in connection with the numerous functions

which are characteristic of the tissues which form the seed coat. In this respect the greatest variability in function is seen in the integument. The cells of the integument which are all epidermal in origin perform protective, digestive and nutritive functions. They store large quantities of food and are later themselves used as food. Other functions of cells derived from the epidermis in *Datura* have been discussed recently in another paper (Satina, 1944). This variability in function, together with the fact that the epidermal cells may change readily their functions at various stages, supports Linsbauer's interpretation of the epidermis (1930). Arguing against the physiological definition of this tissue, according to which the epidermis is only a purely protective tissue, Linsbauer offers a broader morphological concept of the epidermis. He believes that one of the most important differences between the epidermis and other tissues lies in the fact that the epidermis is not limited to one main function, but that it can perform several others. It is the ability to carry on a variety of other functions which is considered by Linsbauer as one of the main properties of the epidermis. The present paper is in complete agreement with the point of view of Linsbauer regarding the diverse functions of the epidermis, which has been found to form not only the outer coating of the ovular coat but also the entire inner tissue of the integument.

SUMMARY

The ovule is initiated from the innermost layer, L III.

The nucellus develops from the median layer, L II.

The integument arises at the level just below the base of the archesporial cell and develops from the outermost layer, L I.

The archesporial cell arises in the subepidermal layer of the nucellus. Nucellar cells surrounding the megaspore are digested and absorbed during the development of the ovule. Nucellar cells in the chalazal portion persist and remain active.

The micropylar portion of the ovular coat is formed by the integument; the chalazal portion is formed by nucellar cells.

DEPARTMENT OF BOTANY,
SMITH COLLEGE,
NORTHAMPTON, MASSACHUSETTS

LITERATURE CITED

- BOWER, F. O. 1919. Botany of the living plants. p. 258. McMillan. London.
- BROWN, MABEL M. 1917. The development of the embryo sac and of the embryo in *Phaseolus vulgaris*. Bull. Torrey Bot. Club 44: 539-544.
- COOK, M. T. 1924. Development of seed of *Linaria vulgaris*. Bot. Gaz. 77: 225-227.
- COOPER, D. C. 1931. Macrosporogenesis and the development of the megagametophyte of *Lycopersicon esculentum*. Amer. Jour. Bot. 18: 739-748.
- COOPER, D. C., AND R. A. BRINK. 1940. Somatoplastic

- sterility as a cause of seed failure after interspecific hybridization. *Genetics* 25:593-617.
- DAHLGREEN, K. V. O. 1927. Die Morphologie des Nuzelus. *Jahrb. wiss. Bot.* 67:347-426.
- . 1928. Die Embryologie einiger Alismatazeen. *Svensk. Bot. Tidskr.* 22:1-17.
- ENGLER, A., AND K. PRANTL. 1926. Die natürlichen Pflanzenfamilien. Angiospermen. 14a. p. 52. Leipzig.
- GLISIC, L. J. M. 1928. Zur Entwicklungsgeschichte der Solanaceae. Die Endospermibildung von *Datura metel*. *Belgr. Un. Bull. Inst. et Jard. Bot.* 1:106-141.
- GOEBEL, K. 1933. Organographie der Pflanzen. III. 3 Aufl. Jena. p. 2003-2005, 2015.
- GUIGNARD, L. 1893. Recherches sur le développement de la graine et en particulier du tégument seminal. *Jour. de Bot.* 7:284.
- . 1902. La double fécondation chez les Solanées. *Jour. de Bot.* 16:145-167.
- HAUPT, A. W. 1933-1934. Ovule and embryo sac of *Plumbago capensis*. *Bot. Gaz.* 95:649-659.
- LESLEY, MARGARET. 1926. Maturation in diploid and triploid tomatoes. *Genetics* 11:267-279.
- LINSBAUER, K. 1930. Die Epidermis. *Handb. d. Pflanzenanatomie*. Bd. 4:1-284. Berlin.
- PEARSON, O. H. 1932-1933. Study of the life history of *Brassica oleracea*. *Bot. Gaz.* 94:534-550.
- REES-LEONARD, O. L. 1935. Macrosporogenesis and development of the megagametophyte of *Solanum tuberosum*. *Bot. Gaz.* 96:734-750.
- SATINA, S., A. F. BLAKESLEE, AND A. AVERY. 1934. Twins in the jimson weed, *Datura stramonium*, *Amer. Nat.* 68:162. (Abstract.)
- , ———, AND ———. 1940. Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. *Amer. Jour. Bot.* 27:895-905.
- SATINA, S., AND A. F. BLAKESLEE. 1935. Cytological effects of a gene in *Datura* which causes dyad formation in sporogenesis. *Bot. Gaz.* 96:521-532.
- , AND ———. 1937. Chromosome behavior in triploid *Datura*. II. The female gametophyte. *Amer. Jour. Bot.* 24:621-627.
- , AND ———. 1941. Periclinal chimeras in *Datura stramonium* in relation to development of leaf and flower. *Amer. Jour. Bot.* 28:862-871.
- , AND ———. 1943. Periclinal chimeras in *Datura* in relation to the development of the carpel. *Amer. Jour. Bot.* 30:453-462.
- . 1944. Periclinal chimeras in *Datura* in relation to the development and structure (A) of style and stigma (B) of calyx and corolla. *Amer. Jour. Bot.* 31:493-502.
- SCHNARF, KARL. 1929. Embryologie der Angiospermen. *Handbuch der Pflanzenanatomie*. 2. Abt. 2 Teil. Berlin.
- . 1931. Vergleichende Embryologie der Angiospermen. p. 175. Berlin.
- SOUÈGES, R. 1907. Développement et structure du tégument seminal chez les Solanacees. *Ann. Sci. Nat. Bot.* 6:1-124.
- STRASBURGER, E. 1930. Textbook of botany. p. 547. McMillan & Co. London.
- SVENSSON, H. G. 1926. Zytologische embryologische Solanazeenstudien. *Svensk. Bot. Tidskr.* 20:420-434.
- YOUNG, W. J. 1923. The formation and degeneration of germ cells in the potato. *Amer. Jour. Bot.* 10:325-335.
- WALKER, RUTH. 1938. Microsporogenesis and embryo development of *Ulmus fulva*. *Bot. Gaz.* 99:592-598.
- V. WETSTEIN, R. 1935. *Handbuch der systematischen Botanik*. p. 449. 4 Aufl. Leipzig-Wien.

A LIST OF CHROMOSOME NUMBERS IN HIGHER PLANTS.

I. ACANTHACEAE TO MYRTACEAE¹

Wray M. Bowden²

RELATIONSHIPS BETWEEN chromosome number and winter hardiness in the higher plants were reported by Bowden (1940a, 1940b). Numerous plant collections were assembled of genera and species native to tropical, subtropical, and temperate zones. In the present paper and in one to follow, cytological data obtained by the methods described by Bowden (1940a) are recorded. Examinations were made of 222 collections of 179 species of angiosperms and one collection for each of two species of gymnosperms. These were distributed in eighty genera in forty-eight families.

MATERIALS AND METHODS.—The plants were

¹ Received for publication October 2, 1944.

² Formerly Research Fellow in Agricultural Biology of the Blandy Experimental Farm, University of Virginia. The writer expresses his appreciation to Dr. Orland E. White, Director of the Blandy Experimental Farm, for helpful criticism and supervision during this investigation; to the collectors and systematic botanists for their cooperation in assembling and identifying the plants; to Dr. L. H. Bailey for reviewing the taxonomic nomenclature; and to Dr. Orland E. White and Dr. J. T. Baldwin, Jr., for reading the manuscript.

grown in experimental plots from seeds, cuttings, seedlings, etc., obtained from botanical gardens, arboreta, and seed firms, from field collections, or from permanent plantings in the nursery and arboretum of The Blandy Experimental Farm. Each collection was checked as carefully as possible to determine its taxonomic identification. In cases which proved to be difficult, herbarium specimens were sent to taxonomists, including Dr. L. H. Bailey, Dr. Alfred Rehder, and Dr. Paul C. Standley. Adequate material of many collections was available after final observations were recorded in 1941, and herbarium specimens were deposited in the Bailey Herbarium, Ithaca, New York. Many of the species listed in this account are well known, and, even though changes in nomenclature may eventually be made, it is considered that the identifications listed will make recognition possible.

To obtain root tips with many mitoses, plants were carefully dug, and, if only a few suitable root tips were available, the root system was pruned and the plant re-set. By careful watering, root tips with

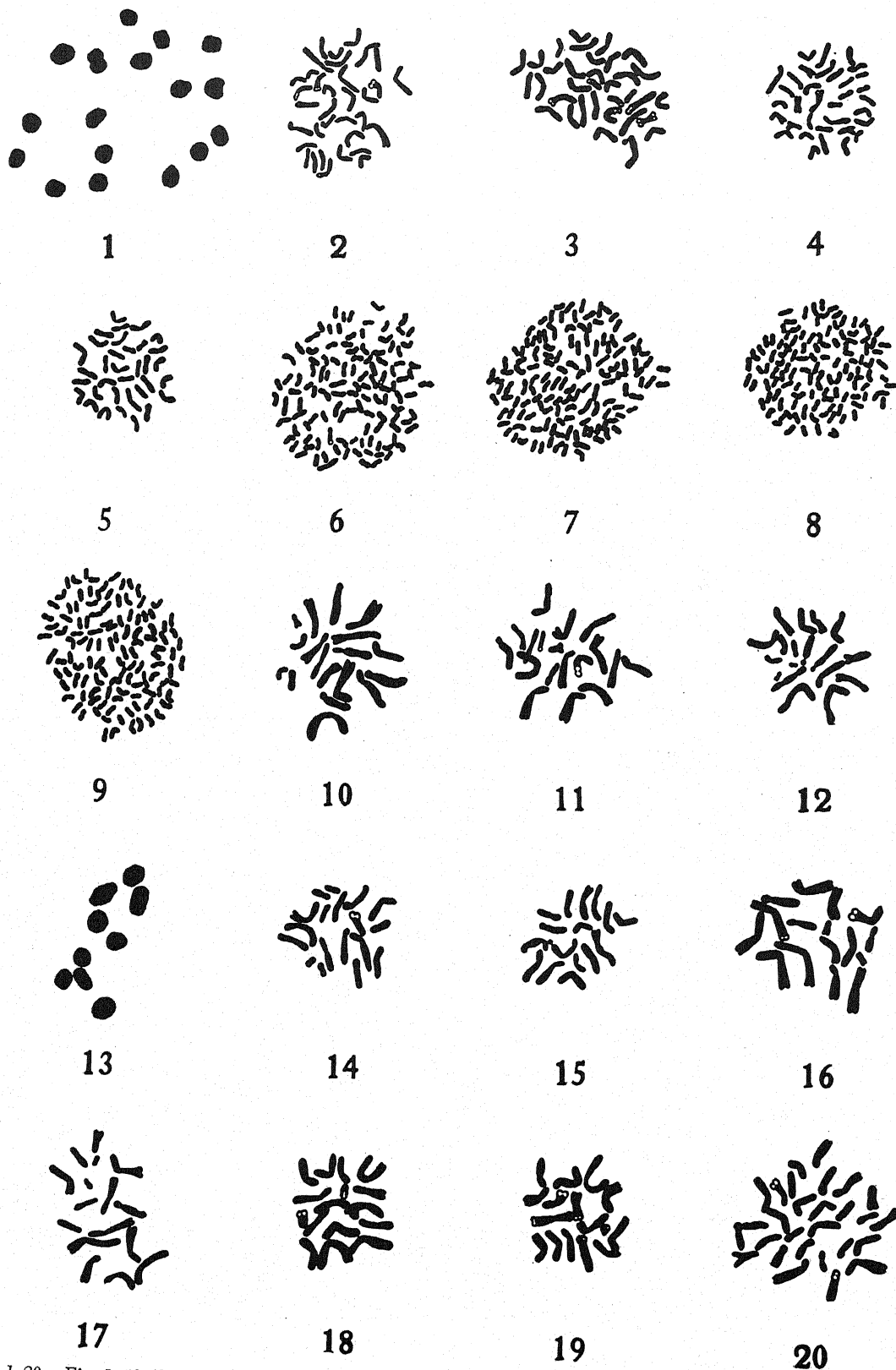


Fig. 1-20.—Fig. 1, 13. Polar views of meiotic stages, magnification $\times 3240$.—Other figures, polar views of mitotic metaphases, magnification $\times 2500$.—Fig. 1-5. Acanthaceae.—Fig. 1. *Ruellia ciliosa* Pursh., Metaphase II, $n=17$.—Fig. 2. *Ruellia malacosperma* Greenm., $2n=34$.—Fig. 3. *Ruellia nudiflora* (Engelm. and Gray.) Urban. var. *occidentalis*

abundant mitoses could usually be obtained within several weeks. Vigorously-growing young seedlings also yielded excellent somatic root-tip material. A few chromosome-number determinations were made from metaphases in somatic tissues of young leaves, stem tips (fig. 85, 99, 100; see also fig. 122, 185, 195, and 196 in the following paper) and young flower buds (fig. 169 and 186 in the following paper); this was found to be especially helpful when only limited plant material was available or when the usual routine methods were unsatisfactory as noted by Bowden (1940b) for *Sassafras albidum* (Nutt.) Nees. var. *molle* (Raf.) Fern.

The preparations of root tips, young leaves, stem tips, and flower buds, were made from material fixed in Webber's modification of Nawashin's fixative, imbedded according to La Cour's alcohol-chloroform-paraffin schedule, sectioned at 10 to 15 μ , and stained in Newton's iodine-gentian-violet or in Heidenhain's iron-alum haematoxylin. Root tips were fixed between 9.00 a.m. and 3.00 p.m. Webber's modification of Nawashin's fixative (similar to Belling's modification of Nawashin's fixative) was valuable in that it yielded relatively uniform results for many different species in various genera and families.

Certain materials proved to be difficult to fix satisfactorily. In such cases, when the large freshly-cut root tips were sliced longitudinally into halves and quarters by means of a sharp razor blade, fixation was much improved, e.g., in species of Annonaceae. Drawings of somatic chromosome complements in root-tip cells were made from metaphases in periblem cells midway between the median axis and the perimeter in the most mitotically-active zone, since these were the largest cells, and the chromosomes were usually better dispersed; an-exception is figure 76 which was drawn from a metaphase in an inner periblem cell. Chromosome numbers were determined in all regions of the somatic tissues in serial sections of root tips, young leaves, stem tips, and flower buds.

To obtain meiotic stages in the pollen mother cells, preparations were made by smearing the fresh anthers on a dry slide and staining at once in Belling's aceto-carmin. Anthers with the desired stages of meiosis were obtained between 10.00 a.m. and 3.00 p.m.

Drawings were made by use of a Zeiss microscope equipped with a 90 X oil immersion objective, N.A. 1.25, and K. 15 X and K. 20 X oculars, in conjunction with an Abbé camera lucida and a level drawing board.

CYTOLOGICAL DATA.—Genera are listed under (Gray.) Leonard., 2n=34.—Fig. 4. *Ruellia strepens* L., 2n=34.—Fig. 5. *Ruellia tuberosa* L., 2n=34.—Fig. 6-9. Actinidiaceae.—Fig. 6. *Actinidia arguta* (Sieb. and Zucc.) Miq., 2n=ca. 116.—Fig. 7. *Actinidia* X *Fairchildii* Rehd., 2n=ca. 132.—Fig. 8. *Actinidia chinensis* Planch., 2n=ca. 116.—Fig. 9. *Actinidia polygama* (Sieb. and Zucc.) Miq., 2n=ca. 116.—Fig. 10-20. Annonaceae.—Fig. 10. *Asimina obovata* (Willd.) Nash., 2n=18.—Fig. 11. *Asimina obovata* (Willd.) Nash. var. *grandiflora* Hort., 2n=18.—Fig. 12. *Asimina parviflora* (Michx.) Dunal., 2n=18.—Fig. 13. *Asimina triloba* (L.) Dunal., Metaphase I, n=9.—Fig. 14. *Asimina triloba* (L.) Dunal., 2n=18.—Fig. 15. *Cananga odorata* Hook. f. and Thoms., 2n=16.—Fig. 16. *Annona montana* Macfad., 2n=16.—Fig. 17. *Annona muricata* L., 2n=16.—Fig. 18. *Annona reticulata* L., 2n=16.—Fig. 19. *Annona squamosa* L., 2n=16.—Fig. 20. *Annona glabra* L., 2n=28.

families which are arranged in approximate alphabetical order; this arrangement results from the grouping on the same plate of the drawings for all the species of one family. Data for each collection include: Blandy Experimental Farm accession number; figure number; taxonomic identification; source of material; chromosome number determined during this investigation; and previously-reported chromosome numbers as cited by Tischler (1927, 1931, 1935, 1936, 1938) or Gaiser (1926, 1930a, 1930b, 1933) or as known to the author from publications of later dates.

The lists found in this and the following paper are a complete record of the cytological examinations made during this investigation and include the one hundred collections previously reported by Bowden (1940a). The chromosome complements of 177 angiospermous species and one gymnospermous species are figured in the two papers by 204 camera-lucida drawings. There are 163 drawings of mitotic metaphases for 147 species, which have a magnification $\times 2500$. Most of the forty-one drawings of meiotic stages (usually Metaphase I) of thirty-seven species have a magnification $\times 3240$ with the exception of a few which have a magnification $\times 2500$, namely: fig. 83, 88, 89, 115, 116 of this paper; fig. 133, 161, 163, 164 of the following paper.

CHROMOSOME NUMBERS.—*Acanthaceae*.—No. 3380-29, fig. 1. *Ruellia ciliosa* Pursh., col. by O. E. White near Riverton, Virginia, n=17 (2n=34, Bowden, 1940a).—No. 5750-39, fig. 2. *Ruellia malacosperma* Greenm., B.G. (i.e., Botanical Garden), Singapore, Straits Settlements, 2n=34.—No. 5751-38, fig. 3. *Ruellia nudiflora* (Engelm. and Gray.) Urban. var. *occidentalis* (Gray.) Leonard., col. by O. E. White near Victoria, Mexico, 2n=34.—No. 5752-39, fig. 4. *Ruellia strepens* L., Rex D. Pearce, N.J., 2n=34.—No. 6760-39, *Ruellia strepens* L., B.G., Leipzig, Germany, 2n=34.—No. 5753-39, fig. 5. *Ruellia tuberosa* L., B.G., Copenhagen, Denmark, 2n=34 (n=16, Sugiura, 1936).

Actinidiaceae.—No. 7276-40, fig. 6. *Actinidia arguta* (Sieb. and Zucc.) Miq., Bay State Nursery, Mass., 2n=ca. 116.—No. 494-35, fig. 7. *Actinidia* X *Fairchildii* Rehd. (= *A. arguta* (Sieb. and Zucc.) Miq. ♀ X *A. chinensis* Planch. ♂), Bureau Plant Introduction, P.I. No. 92303, staminate plant, 2n=ca. 132.—No. 495-33, fig. 8. *Actinidia chinensis* Planch., Arnold Arboretum, Mass., 2n=ca. 116.—No. 496-34, fig. 9. *Actinidia polygama* (Sieb. and Zucc.) Miq., staminate plant, Bobbink and Atkins, N.J., 2n=ca. 116.

Amaryllidaceae.—No. 508-36, *Manfreda virgin-*



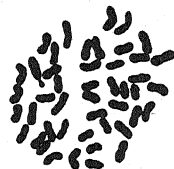
21



22



23



24



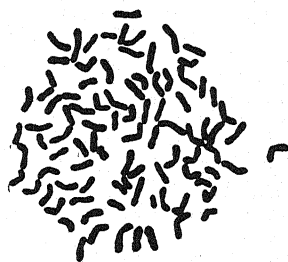
25



26



27



28



29



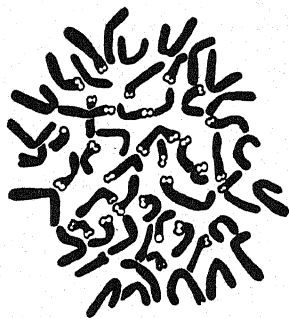
30



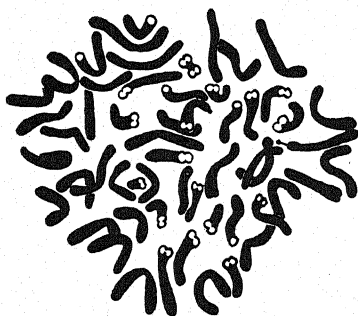
31



32



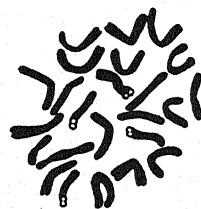
33



34



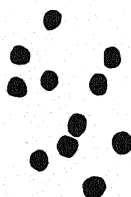
35



36



37



38



39



40

Fig. 21-40.

(See page 89 for description of figures.)

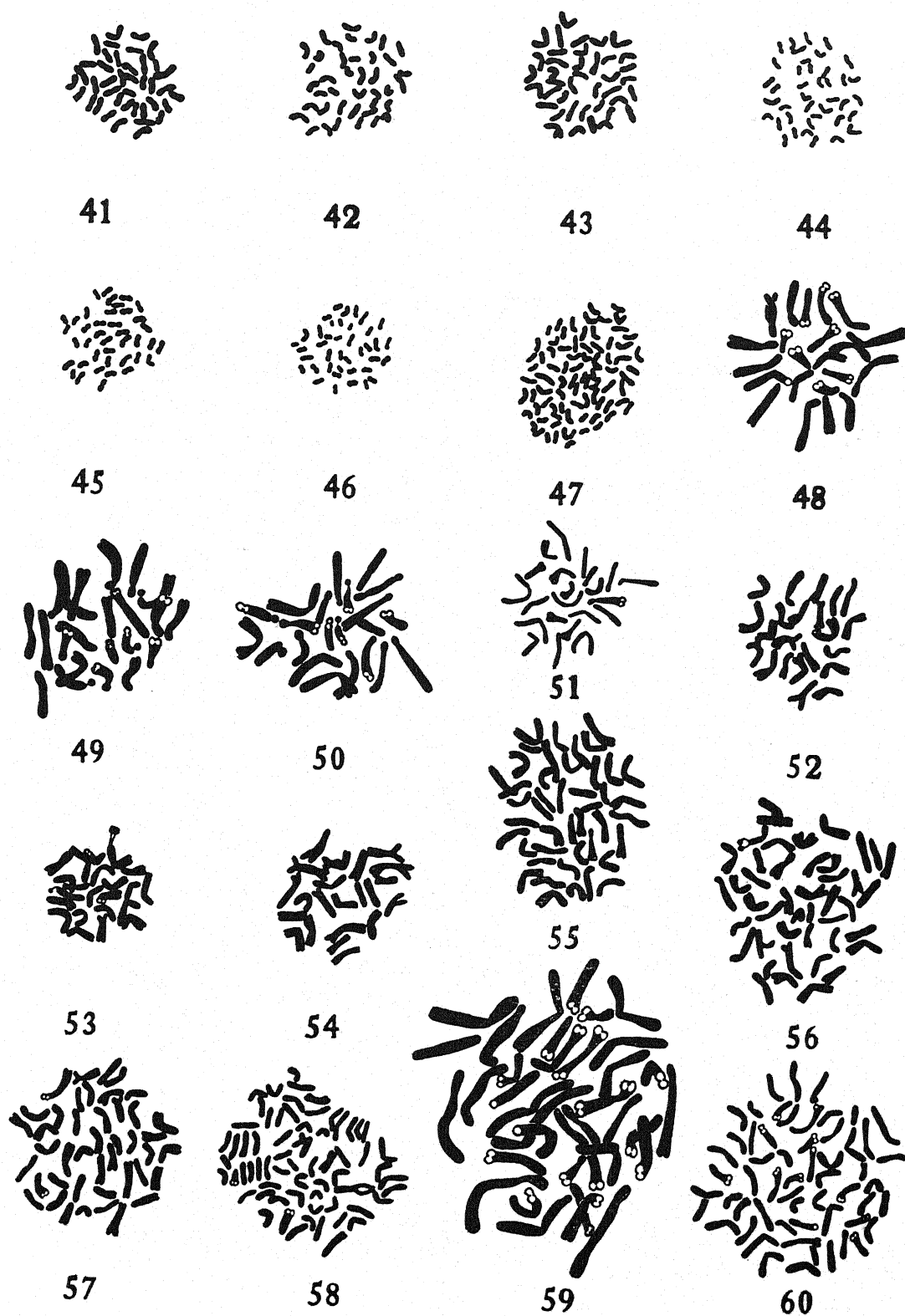
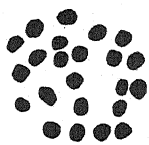
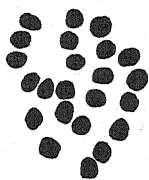


Fig. 41-60.

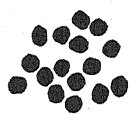
(See page 89 for description of figures.)



61



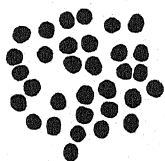
62



63



64



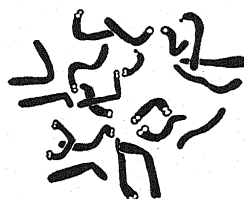
65



66



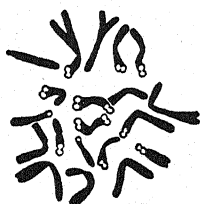
67



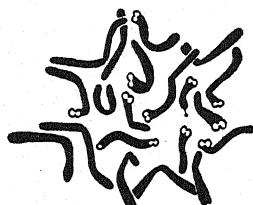
68



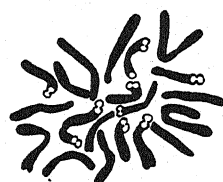
69



70



71



72



73



74



75



76



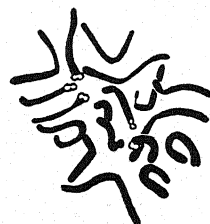
77



78



79



80

Fig. 61-80.

(See page 89 for description of figures.)



81



82



83



84



85



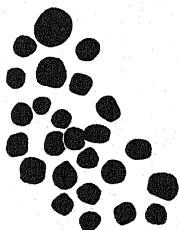
86



87



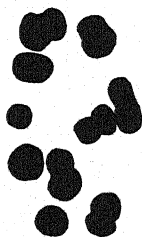
88



89



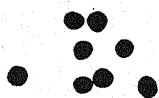
90



91



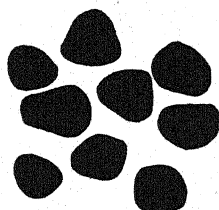
92



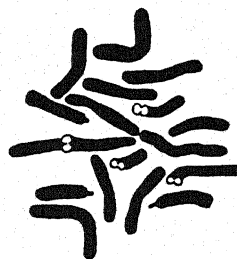
93



94



95



96



97



98



99



100

Fig. 81-100.

(See page 89 for description of figures.)



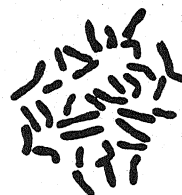
101



102



103



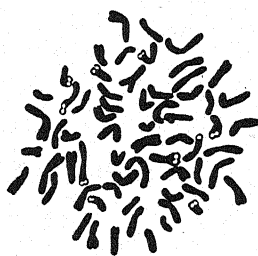
104



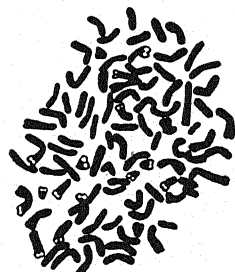
105



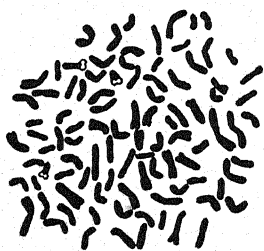
106



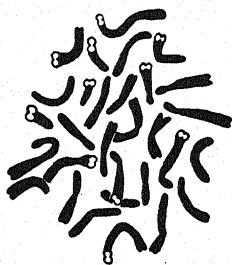
107



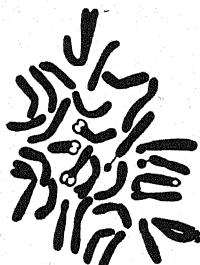
108



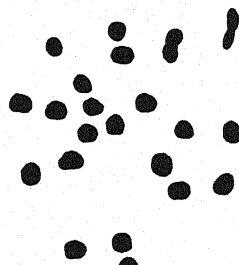
109



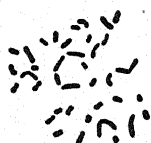
110



111



112



113



114



115



116



117



118



119



120

Fig. 101-120.

(See page 89 for description of figures.)

ica (L.) Salisb., Boyce Thompson Arboretum, Yonkers, New York, $n=30$ ($2n=60$, McKelvey and Sax, 1933) ($n=12$, Schaffner, 1909).

Annonaceae.—No. 7601–40, fig. 10. *Asimina obovata* (Willd.) Nash., E. West, Gainesville, Florida, $2n=18$.—No. 7593–40, fig. 11. *Asimina obovata* (Willd.) Nash. var. *grandiflora* Hort., G. A. Zim-

merman, Harrisburg, Pa., $2n=18$.—No. 7600–40, fig. 12. *Asimina parviflora* (Michx.) Dunal, col. by W. C. Coker, Chapel Hill, North Carolina, $2n=18$.—586–26, fig. 13. *Asimina triloba* (L.) Dunal, collection at The Blandy Experimental Farm from New York, Kentucky, Maryland, Michigan, Ohio, and Indiana, $n=9$; ($n=9$, Locke, 1936).—No. 586–

Fig. 21–40.—Fig. 21, 38. Polar views of Metaphase I, magnification $\times 3240$.—Other figures, polar views of mitotic metaphases, magnification $\times 2500$.—Fig. 21–29. Apocynaceae.—Fig. 21. *Lochnera rosea* (L.) Reichb., Metaphase I, $n=8$.—Fig. 22. *Vinca difformis* Pourr., $2n=46$.—Fig. 23. *Vinca herbacea* Waldst. and Kit., $2n=46$.—Fig. 24. *Vinca minor* L., $2n=46$.—Fig. 25. *Vinca minor* L. var. *alba* West., $2n=46$.—Fig. 26. *Vinca minor* L. var. *Bowles* Hort., $2n=46$.—Fig. 27. *Vinca minor* L. var. *flore-pleno purpurea* Hort., $2n=46$.—Fig. 28. *Vinca major* L., $2n=92$.—Fig. 29. *Vinca major* L. var. *variegata* Loud., $2n=92$.—Fig. 30–34. Araceae.—Fig. 30. *Arisaema ambiguum* Engl., $2n=26$.—Fig. 31. *Arisaema quinatum* (Nutt.) Schott., $2n=28$.—Fig. 32. *Arisaema concinnum* Schott., $2n=56$.—Fig. 33. *Arisaema Dracontium* (L.) Schott., $2n=56$.—Fig. 34. *Arisaema triphyllum* (L.) Schott., $2n=56$.—Fig. 35–37. Araliaceae.—Fig. 35. *Aralia nudicaulis* L., $2n=24$.—Fig. 36. *Aralia spinosa* L., $2n=24$.—Fig. 37. *Aralia californica* Wats., $2n=48$.—Fig. 38. Asclepiadaceae.—Fig. 38. *Periploca graeca* L., Metaphase I, $n=11$.—Fig. 39, 40. Begoniaceae.—Fig. 39. *Begonia Evansiana* Andr., $2n=24$.—Fig. 40. *Begonia cucullata* Willd., $2n=56$.

Fig. 41–60.—Polar views of mitotic metaphases, magnification $\times 2500$.—Fig. 41–51. Bignoniaceae.—Fig. 41. *Bignonia capreolata* L., $2n=40$.—Fig. 42. *Campsis grandiflora* (Thunb.) Loisel. var. *Mme. Gallen* Hort., $2n=40$.—Fig. 43. *Chilopsis linearis* (Cav.) Sweet., $2n=40$.—Fig. 44. *Kigelia pinnata* DC., $2n=40$.—Fig. 45. *Stenolobium stans* Seem., $2n=40$.—Fig. 46. *Stenolobium stans* Seem. var. *angustata* Rehd., $2n=40$.—Fig. 47. *Doxantha Unguis-cati* Rehd., $2n=80$.—Fig. 48. *Incarvillea compacta* Maxim., $2n=22$.—Fig. 49. *Incarvillea Olgae* Regel., $2n=22$.—Fig. 50. *Incarvillea grandiflora* Bur. and Franch., $2n=22$.—Fig. 51. *Incarvillea Olgae* Regel., $2n=22$.—Fig. 52–58. Cactaceae.—Fig. 52. *Opuntia arborescens* Engelm., $2n=22$.—Fig. 53. *Opuntia repens* Bello., $2n=22$.—Fig. 54. *Opuntia compressa* Macbride., $2n=22$.—Fig. 55. *Opuntia compressa* Macbride., $2n=44$.—Fig. 56. *Opuntia impedita* Small., $2n=44$.—Fig. 57. *Opuntia salmiana* Parm., $2n=44$.—Fig. 58. *Opuntia fragilis* Haw., $2n=66$.—Fig. 59. Caprifoliaceae.—Fig. 59. *Sambucus javanica* Reinw., $2n=36$.—Fig. 60. Commelinaceae.—Fig. 60. *Commelina hirtella* Vahl., $2n=ca. 58$.

Fig. 61–80.—Fig. 61, 62, 63, 64, 65. Polar views of Metaphase I, magnification $\times 3240$.—Fig. 66–80. Polar views of mitotic metaphases, magnification $\times 2500$.—Fig. 61–66. Celastraceae.—Fig. 61. *Celastrus orbiculata* Thunb., Metaphase I, $n=23$.—Fig. 62. *Celastrus scandens* L., Metaphase I, $n=23$.—Fig. 63. *Euonymus japonica* L., Metaphase I, $n=16$.—Fig. 64. *Euonymus Fortunei* (Turcz.) Hand.-Mazz. var. *vegeta* (Rehd.) Rehd., Metaphase I, $n=16$.—Fig. 65. *Euonymus americana* L., Metaphase I, $n=32$.—Fig. 66. *Pachistima Canbyi* Gray., $2n=32$.—Fig. 67–80. Cistaceae.—Fig. 67. *Helianthemum alpestre* (Jacq.) Dun., $2n=20$.—Fig. 68. *Helianthemum* sp., $2n=20$.—Fig. 69. *Helianthemum glaucum* (Cav.) Pers., $2n=20$.—Fig. 70. *Helianthemum apenninum* (L.) Mill., $2n=20$.—Fig. 71. *Helianthemum ovatum* Dun., $2n=20$.—Fig. 72. *Helianthemum pilosum* Mill., $2n=20$.—Fig. 73. *Helianthemum polifolium* Mill., $2n=20$.—Fig. 74. *Helianthemum apenninum* (L.) Mill., $2n=20$.—Fig. 75. *Helianthemum roseum* DC., $2n=20$.—Fig. 76. *Helianthemum canum* Boiss., $2n=22$.—Fig. 77. *Helianthemum nummularium* Mill., $2n=32$.—Fig. 78. *Cistus albidus* L., $2n=18$.—Fig. 79. *Cistus X hybridus* Pourr., $2n=18$.—Fig. 80. *Cistus laurifolius* L., $2n=18$.

Fig. 81–100.—Fig. 83, 88, 89, 91, 93, 94, 95. Polar views of meiotic stages. Other figures, polar views of mitotic metaphases.—Magnification $\times 2500$, except fig. 91, 93, 94, 95, which have a magnification $\times 3240$.—Fig. 81, 82. Cistaceae.—Fig. 81. *Cistus symphytifolius* Lam., $2n=18$.—Fig. 82. *Cistus villosus* L. var. *tauricus* (Presl.) Grosser., $2n=18$.—Fig. 83–89. Compositae.—Fig. 83. *Baccharis pinnata* DC., Metaphase I, $n=9$.—Fig. 84. *Baccharis genistelloides* Pers., $2n=18$.—Fig. 85. *Baccharis genistifolia* DC., $2n=18$.—Fig. 86. *Baccharis halimifolia* L., $2n=18$.—Fig. 87. *Baccharis phyteumoides* DC., $2n=18$.—Fig. 88. *Gerberia Jamesonii* Bolus. var. *hybrida* Hort., Metaphase II, $n=25$.—Fig. 89. *Gerberia Jamesonii* Bolus var. *hybrida* Hort., Metaphase I, $n=25$.—Fig. 90. Coriariaceae.—Fig. 90. *Coriaria myrtifolia* L., $2n=ca. 80$.—Fig. 91, 92. Euphorbiaceae.—Fig. 91. *Euphorbia Lathyris* L., Metaphase I, $n=10$.—Fig. 92. *Manihot palmata* (Vell.) Pax., $2n=36$.—Fig. 93, 94. Fumariaceae.—Fig. 93. *Corydalis sempervirens* (L.) Pers., Metaphase I, $n=8$.—Fig. 94. *Dicentra eximia* Torr., Metaphase I, $n=8$.—Fig. 95, 96. Hydrophyllaceae.—Fig. 95. *Hydrophyllum canadense* L., Metaphase I, $n=9$.—Fig. 96. *Hydrophyllum virginianum* L., $2n=18$.—Fig. 97. Juglandaceae.—Fig. 97. *Juglans insularis* Griseb., $2n=32$.—Fig. 98–100. Lauraceae.—Fig. 98. *Persea americana* Mill., $2n=24$.—Fig. 99. *Persea palustris* (Raf.) Sarg., $2n=24$.—Fig. 100. *Sassafras albidum* (Nutt.) Nees. var. *molle* (Raf.) Fern., $2n=48$.

Fig. 101–120.—Fig. 112, 115, 116. Polar views of Metaphase I.—Other figures, polar views of mitotic metaphases.—Magnification $\times 2500$, except fig. 112 which has a magnification $\times 3240$.—Fig. 101–109. Iridaceae.—Fig. 101. *Sisyrinchium* sp., $2n=16$.—Fig. 102. *Sisyrinchium iridifolium* H.B. and K., $2n=18$.—Fig. 103. *Sisyrinchium albidum* Raf., $2n=32$.—Fig. 104. *Sisyrinchium bellum* Wats., $2n=32$.—Fig. 105. *Sisyrinchium brachypus* (Bickn.) J. K. Henry., $2n=36$.—Fig. 106. *Sisyrinchium californicum* Dryand., $2n=36$.—Fig. 107. *Sisyrinchium* sp., $2n=64$.—Fig. 108. *Sisyrinchium* sp., $2n=90$.—Fig. 109. *Sisyrinchium angustifolium* Mill., $2n=96$.—Fig. 110–114. Liliaceae.—Fig. 110. *Anthericum Liliago* L., $2n=30$.—Fig. 111. *Anthericum Liliastrium* L. var. *major* Hort., $2n=30$.—Fig. 112. *Arthropodium cirrhatum* R. Br., Metaphase I, $n=22$.—Fig. 113. *Cordylina australis* Hook. f., $2n=38$.—Fig. 114. *Dracaena Draco* L., $2n=38$.—Fig. 115, 116. Lobeliaceae.—Fig. 115. *Lobelia Cardinalis* L. Subsp. *Cardinalis* McVaugh., Metaphase I, $n=7$.—Fig. 116. *Lobelia Cardinalis* L. subsp. *graminea* (Lam.) McVaugh. var. *pseudosplendens* McVaugh., Metaphase I, $n=7$.—Fig. 117, 118. Lythraceae.—Fig. 117. *Lagerstroemia indica* L. var. *rubra* Hort., $2n=50$.—Fig. 118. *Lagerstroemia speciosa* Pers., $2n=50$.—Fig. 119. Meliaceae.—Fig. 119. *Melia Azedarach* L., $2n=28$.—Fig. 120. Myrtaceae.—Fig. 120. *Feijoa Sellowiana* Berg., $2n=22$.

26, fig. 14. *Asimina triloba* (L.) Dunal, same as preceding collection, $2n=18$.—No. 7596–40, fig. 15. *Cananga odorata* Hook. f. and Thoms., S. J. Lynch, Homestead, Fla., $2n=16$.—7599–40, fig. 16. *Annona montana* Macfad., B.G., Buitenzorg, Java, $2n=16$.—No. 6848–39, fig. 17. *Annona muricata* L., B.G., Berlin-Dahlem, Germany, $2n=16$.—No. 7594–40, fig. 18. *Annona reticulata* L., S. J. Lynch, Homestead, Fla., $2n=16$.—No. 7595–40, fig. 19. *Annona squamosa* L., G. A. Zimmerman, Harrisburg, Pa., $2n=16$.—No. 7592–40, fig. 20. *Annona glabra* L., G. A. Zimmerman, Harrisburg, Pa., $2n=28$.

Apocynaceae.—No. 5746–38, fig. 21. *Lochnera rosea* (L.) Reichb., Northrup, King and Co., Minn., $n=8$ ($n=8$, Margadant, 1927, Sugiura, 1936, Pannocchia-Laj, 1938, Schnell, 1941) ($2n=16$, Bowden, 1940a, Sugiura, 1931, and Pannocchia-Laj, 1938).—No. 7529–40, fig. 22. *Vinca difformis* Pourr., B.G., Coimbra, Portugal, $2n=46$ ($2n=46$, Pannocchia-Laj, 1938).—No. 2771–39, fig. 23. *Vinca herbacea* Waldst. and Kit., Hardy Plant Nursery, Dropmore, Man., $2n=46$; ($n=23$, Finn, 1928).—No. 3946–34, fig. 24. *Vinca minor* L., O. E. White, Charlottesville, Va., $2n=46$ ($n=23$, Finn, 1928); ($2n=46$, Pannocchia-Laj, 1938) ($n=16$, Schürhoff and Müller, 1937).—No. 3947–34, fig. 25. *Vinca minor* L. var. *alba* West., Henry A. Dreer, Pa., $2n=46$.—No. 3948–34, fig. 26. *Vinca minor* L. var. *Bowles* Hort., Henry A. Dreer, Pa., $2n=46$.—No. 3944–35, fig. 27. *Vinca minor* L. var. *flore-pleno purpurea* Hort., Carl Purdy, Calif., $2n=46$.—No. 7555–38, fig. 28. *Vinca major* L., Valley View Nursery, Virginia, $2n=92$ ($2n=92$, Pannocchia-Laj, 1938); ($n=8$, Schürhoff and Müller, 1937).—No. 6481–40, *Vinca major* L., Palace Gardens, Williamsburg, Va., $2n=92$.—6467–37, *Vinca major* L., Old Estate, "Plain Dealing," near Scottsville, Va., $2n=92$.—No. 7197–40, fig. 29. *Vinca major* L. var. *variegata* Loud., B.G., Ann Arbor, Mich., $2n=92$.—No. 7074–40, *Vinca major* L. var. *variegata* Loud., Holland's Nursery, Charlottesville, Va., $2n=92$.

Araceae.—No. 6858–40, fig. 30. *Arisaema ambiguum* Engl., B.P.I., P.I. No. 119220, col. near Kulu, Punjab, India, $2n=26$.—No. 5707–39, fig. 31. *Arisaema quinatum* (Nutt.) Schott., col. in Great Smokey Mountains, Tennessee, through B.G., Ann Arbor, Mich., $2n=28$.—No. 5705–38, fig. 32. *Arisaema concinnum* Schott., Lloyd B.G., Darjeeling, India, $2n=56$.—No. 5706–39, fig. 33. *Arisaema Dracontium* (L.) Schott., Kohankie and Sons, Ohio, $2n=56$.—No. 5708–38, fig. 34. *Arisaema triphyllum* (L.) Schott., col. by W. M. Bowden in Clarke County, Virginia, $2n=56$ ($n=16$, Atkinson, 1899).—No. 6634–39, *Arisaema triphyllum* (L.) Schott., col. by W. M. Bowden at Turkey Point, Norfolk County, Ontario, $2n=56$.

Araliaceae.—No. 6595–40, fig. 35. *Aralia nudicaulis* L., Williams Nursery Co., New Hampshire, $2n=24$.—No. 6825–39, fig. 36. *Aralia spinosa* L., col. by W. M. Bowden in the University of Virginia

forest, Charlottesville, Va., $2n=24$.—No. 6588–40, fig. 37. *Aralia californica* Wats., Carl Purdy, Calif., $2n=48$.

Asclepiadaceae.—No. 3000–35, fig. 38. *Periploca graeca* L., Boyce Thompson Arb., N. Y., $n=11$ ($n=12$, Pardi, 1933).

Begoniaceae.—No. 7751–39, fig. 39. *Begonia Evansiana* Andr., O. E. White, Charlottesville, Va., $2n=24$ ($2n=26$, Matsuura and Okuno, 1936).—No. 6647–38, fig. 40. *Begonia cucullata* Willd., B.G., Montevideo, Uruguay, $2n=56$.

Bignoniaceae.—No. 5709–38, fig. 41. *Bignonia capreolata* L., col. by O. E. White near Chapel Hill, North Carolina, $2n=40$.—No. 667–34, fig. 42. *Campsis grandiflora* (Thunb.) Loisel. var. *Mme. Gallen* Hort., Bobbink and Atkins, N.J., $2n=40$ ($n=20$, Sax, 1933); ($n=18$, Sugiura, 1935).—No. 2108–39, fig. 43. *Chilopsis linearis* (Cav.) Sweet., Soil Conservation Service, Oklahoma, col. at Woodward, Okla., $2n=40$.—No. 804–29, *Chilopsis linearis* (Cav.) Sweet., Munson Nursery, Texas, $2n=40$.—No. 2388–37, fig. 44. *Kigelia pinnata* DC., Atkins Inst., Arnold Arboretum, Cuba, $2n=40$.—No. 271–38, fig. 45. *Stenolobium stans* Seem., Atkins Inst., Arnold Arboretum, Cuba, $2n=40$.—No. 5754–39, fig. 46. *Stenolobium stans* Seem. var. *angustata* Rehd., col. by W. S. Flory, west of the Pecos River, Texas, $2n=40$.—No. 6721–39, fig. 47. *Doxantha Unguis-cati* Rehd., B.G., Coimbra, Portugal, $2n=80$.—No. 5738–39, fig. 48. *Incarvillea compacta* Maxim., B.G., Stockholm, Sweden, $2n=22$.—No. 5739–38, fig. 49. *Incarvillea Olgae* Regel., G. W. Park, S.C., $2n=22$.—No. 5740–38, fig. 50. *Incarvillea grandiflora* Bur. and Franch., Rex. D. Pearce, N.J., $2n=22$ ($2n=18$, Sugiura, 1931).—No. 5471–39, fig. 51. *Incarvillea Olgae* Regel., B.G., Copenhagen, Denmark, $2n=22$.

Cactaceae.—No. 2919–35, fig. 52. *Opuntia arborescens* Engelm., Anderson's Nursery, Arlington, Va., and originally col. near Cheyenne, Wyo., $2n=22$.—No. 2835–39, fig. 53. *Opuntia repens* Bello., Royal Palm Nursery, Oneco, Fla., $2n=22$.—No. 4319–38, fig. 54. *Opuntia compressa* Macbride., col. by W. M. Bowden near Southport, North Carolina, $2n=22$.—No. 4320–28, fig. 55. *Opuntia compressa* Macbride., col. by O. E. White near Riverton, Virginia, $2n=44$.—No. 4317–38, fig. 56. *Opuntia impedita* Small., col. by W. M. Bowden at Wrightsville Beach, North Carolina, $2n=44$.—No. 2836–39, fig. 57. *Opuntia Salmiana* Parm., Royal Palm Nursery, Oneco, Florida, $2n=44$.—No. 2769–39, fig. 58. *Opuntia fragilis* Haw., Hardy Plant Nursery, Manitoba, $2n=66$.

Caprifoliaceae.—No. 5626–38, fig. 59. *Sambucus javanica* Reinw., Lloyd B.G., Darjeeling, India, $2n=36$.

Commelinaceae.—No. 5717–38, fig. 60. *Commelina hirtella* Vahl., col. by O. E. White in North Carolina, $2n=ca. 58$.

Celastraceae.—No. 761–34, fig. 61. *Celastrus orbiculata* Thunb., O. E. White, Charlottesville, Va.,

$n=23$; ($2n=16$, Bowden, 1940a) ($n=23$, Nakajima, 1933).—No. 763–35, fig. 62. *Celastrus scandens* L., staminate plant, native, Blandy Experimental Farm, Clarke County, Virginia., $n=23$.—No. 1206–28, fig. 63. *Euonymus japonica* L., Cora Lee Young Nursery, N.C., $n=16$ ($n=16$, Sugiura, 1931, Morinaga and Fukushima, 1931).—No. 1217–31, fig. 64. *Euonymus Fortunei* (Turcz.) Hand.-Mazz. var. *vegeta* (Rehd.) Rehd., Henry A. Dreer, Pa., $n=16$.—No. 5722–34, fig. 65. *Euonymus americana* L., col. by O. E. White, native, Albemarle County, Virginia., $n=32$.—No. 2946–34, fig. 66. *Pachistima Canbyi* Gray., Bobbink and Atkins, N.J., $2n=32$.

Cistaceae.—No. 5725–38, fig. 67. *Helianthemum alpestre* (Jacq.) Dun., B.G., Geneva, Switzerland, $2n=20$ ($n=16$, Chiarugi, 1925).—No. 5726–38, fig. 68. *Helianthemum* sp., B.G., Geneva, Switzerland, $2n=20$.—No. 5728–38, fig. 69. *Helianthemum glaucum* (Cav.) Pers., B.G., Geneva, Switzerland, $2n=20$.—No. 5729–38, fig. 70. *Helianthemum apenninum* (L.) Mill., B.G., Geneva, Switzerland, $2n=20$.—No. 5730–38, fig. 71. *Helianthemum ovatum* Dun., B.G., Geneva, Switzerland, $2n=20$.—No. 5731–38, fig. 72. *Helianthemum pilosum* Mill., B.G., Geneva, Switzerland, $2n=20$.—No. 5732–38, fig. 73. *Helianthemum polifolium* Mill., B.G., Geneva, Switzerland, $2n=20$.—No. 5733–39, fig. 74. *Helianthemum apenninum* (L.) Mill., B.G., Brussels, Belgium, $2n=20$.—No. 5734–39, fig. 75. *Helianthemum roseum* DC., B.G., Berlin-Dahlem, Germany, $2n=20$.—No. 5727–38, fig. 76. *Helianthemum canum* Boiss., B.G., Geneva, Switzerland, $2n=22$.—No. 5735–39, fig. 77. *Helianthemum nummularium* Mill. (= *H. vulgare* Gaertn.), B.G., Edinburgh, Scotland, $2n=32$ ($n=16$, Chiarugi, 1925).—No. 5712–38, fig. 78. *Cistus albidus* L., B.G., Copenhagen, Denmark, $2n=18$ ($n=9$, Chiarugi, 1925).—No. 2734–39, fig. 79. *Cistus X hybridus* Pourr. (= *C. salvifolius* L. X *C. populifolius* L.), W. B. Clarke and Co., Calif., $2n=18$.—No. 5713–38, fig. 80. *Cistus laurifolius* L., B.G., Copenhagen, Denmark, $2n=18$ ($n=9$, Chiarugi, 1925).—No. 5714–38, fig. 81. *Cistus symphytifolius* Lam., B.G., Copenhagen, Denmark, $2n=18$ ($2n=18$, Chiarugi, 1937).—No. 5716–38, fig. 82. *Cistus villosus* L. var. *tauricus* (Presl.) Grosser., B.G., Copenhagen, Denmark, $2n=18$.—No. 5715–38, *Cistus villosus* L., B.G., Copenhagen, Denmark, $2n=18$ ($n=9$, Chiarugi, 1925).

Compositae.—No. 3750–39, fig. 83. *Baccharis pingraea* DC., B.G., Montevideo, Uruguay, $n=9$.—No. 3753–39, fig. 84. *Baccharis genistelloides* Pers., B.G., Montevideo, Uruguay, $2n=18$.—No. 3745–39, fig. 85. *Baccharis genistifolia* DC., B.G., Montevideo, Uruguay, $2n=18$.—No. 6826–39, fig. 86. *Baccharis halimifolia* L., col. by W. M. Bowden at Wrightsville Beach, North Carolina, $2n=18$.—No. 3749–39, fig. 87. *Baccharis phyteumoides* DC., B.G., Montevideo, Uruguay, $2n=18$.—No. 7752–40, fig. 88. *Gerberia Jamesonii* Bolus. var. *hybrida* Hort.,

Valley View Nursery, Va., $n=25$ ($n=25$, $2n=50$, Kishimoto, 1940).—No. 1355–34, fig. 89. *Gerberia Jamesonii* Bolus. var. *hybrida* Hort., hardy form selected from a group of seedlings from Valley View Nursery, Va., by Dr. Orland E. White, $n=25$.—No. 6563–39, *Gerberia Jamesonii* Bolus var. *hybrida* Hort., seedlings from Dr. White's hardy selection (No. 1355–34), $2n=50$.

Coriariaceae.—No. 5719–39, fig. 90. *Coriaria myrtifolia* L., B.G., Coimbra, Portugal, $2n=ca. 80$ ($n=ca. 40$, $2n=ca. 80$, Grimm, 1912).

Euphorbiaceae.—No. 1232–33, fig. 91. *Euphorbia Lathyris* L., col. by O. E. White near the Skyline Drive, at Thornton's Gap, Virginia, $n=10$ ($2n=20$, Perry, 1943).—No. 5749–39, fig. 92. *Manihot palmata* (Vell.) Pax., B.G., Montevideo, Uruguay, $2n=36$ ($2n=36$, Perry, 1943) ($2n=4$, Boiteau, 1938).

Fumariaceae.—No. 5720–37, fig. 93. *Corydalis sempervirens* (L.) Pers., col. by O. E. White near the Skyline Drive, Va., $n=8$.—No. 1046–35, fig. 94. *Dicentra eximia* Torr., E. C. Robbins Nursery, N.C., $n=8$.

Hydrophyllaceae.—No. 5736–37, fig. 95. *Hydrophyllum canadense* L., col. by J. T. Baldwin, Jr., on Cheat Mt., West Virginia, $n=9$ ($n=9$, Winkler, 1921); ($n=12$, Svensson, 1925).—No. 5737–39, fig. 96. *Hydrophyllum virginianum* L., native to The Blandy Experimental Farm, Boyce, Clarke County, Va., $2n=18$.

Juglandaceae.—No. 308–38, fig. 97. *Juglans insularis* Griseb., Atkins Inst., Arnold Arboretum, Cuba, $2n=32$.

Lauraceae.—No. 7754–39, fig. 98. *Persea americana* Mill., Fruit store, Charlottesville, Va., $2n=24$ ($2n=24$, Van Elden, cited by Merrill, 1930).—No. 6854–39, fig. 99. *Persea palustris* (Raf.) Sarg., col. by O. E. White, near Wilmington, North Carolina, $2n=24$.—No. 7182–40, fig. 100. *Sassafras albidum* (Nutt.) Nees. var. *molle* (Raf.) Fern., native, Clarke County, Va., $2n=48$.

Iridaceae.—No. 3773–39, fig. 101. *Sisyrinchium* sp., received as *S. chilense*, B.G., Montevideo, Uruguay, $2n=16$.—No. 6696–39, fig. 102. *Sisyrinchium iridifolium* H.B. and K., Rex. D. Pearce, N.J., $2n=18$.—No. 2779–39, fig. 103. *Sisyrinchium albidum* Raf., col. by W. M. Bowden near Lynchburg, Virginia, $2n=32$.—No. 6589–40, fig. 104. *Sisyrinchium bellum* Wats., Carl Purdy, Calif., $2n=32$ ($n=16$ in four ecotypes, Clausen, Keck, and Hiesey, 1940).—No. 6716–39, fig. 105. *Sisyrinchium brachypus* (Bickn.) J. K. Henry., B.G., Copenhagen, Denmark, $2n=36$.—No. 6590–40, fig. 106. *Sisyrinchium californicum* Dryand., Carl Purdy, Calif., $2n=36$.—No. 6740–39, fig. 107. *Sisyrinchium* sp., received as *S. chilense*, B.G., Leiden, The Netherlands, $2n=64$.—No. 7201–39, fig. 108. *Sisyrinchium* sp., Thompson and Morgan, Ipswich, England, $2n=90$.—No. 7504–40, fig. 109. *Sisyrinchium angustifolium* Mill., col. by W. M. Bowden at Turkey Point, Norfolk County, Ont., $2n=96$.

Liliaceae.—No. 5704–39, fig. 110. *Anthericum Liliago* L., Lissadell, Sligo, I.F.S., $2n=30$ ($n=32$, Elvers, 1932).—No. 545–35, fig. 111. *Anthericum Liliastrum* L. var. *major* Hort., Henry A. Dreer, Pa., $2n=30$; ($n=16$, Stenar, 1928).—No. 1102–38, fig. 112. *Arthropodium cirrhatum* R.Br., B.P.I., P.I. No. 77543, $n=22$ ($n=22$, Hair, 1942).—No. 5718–39, fig. 113. *Cordyline australis* Hook. f. (= *Dracaena australis* Forst.), B.G., Coimbra, Portugal, $2n=38$ ($n=60$, Matsuura and Sutô, 1935).—No. 1904–38, fig. 114. *Dracaena Draco* L., B.G., Lisbon, Portugal, $2n=38$.—No. 1495–37, *Hosta Fortunei* Bailey. var. *gigantea* Bailey., D. M. Andrews, Boulder, Colorado, $n=30$ ($n=30$, Yasui, 1935).

Lobeliaceae.—No. 353–36, fig. 115. *Lobelia Cardinalis* L. subsp. *Cardinalis* McVaugh., col. by O. E. White at the northern end of the Shenandoah Valley, Virginia, $n=7$ ($n=7$, de Vilmorin and Simonet, 1927, Sugiura, 1936, Cooper, 1942) ($2n=14$, Cooper, 1942).—No. 6874–40, fig. 116. *Lobelia Cardinalis* L. subsp. *graminea* (Lam.) McVaugh. var. *pseudosplendens* McVaugh., B.P.I., P.I. No. 133415, col. Ropes Springs, New Mexico, $n=7$.

Lythraceae.—No. 2531–28, fig. 117. *Lagerstroemia indica* L. var. *rubra* Hort., Cora Lee Young Nursery, N.C., $2n=50$.—No. 2532–37, fig. 118. *Lagerstroemia speciosa* Pers., Atkins Inst., Arnold Arboretum, Cuba, $2n=50$.

Meliaceae.—No. 136–38, fig. 119. *Melia Azedarach* L., B.G., Darjeeling, India, col. at 3,000 ft., $2n=28$.—No. 4266–36, *Melia Azedarach* L., var. *umbraculiformis* Berckmans., col. by O. E. White near Wake Forest, North Carolina, $2n=28$.

Myrtaceae.—No. 5723–38, fig. 120. *Feijoa Selowiana* Berg., B.G., Montevideo, Uruguay, $2n=22$.

SUMMARY

Chromosome numbers are presented and figured for a large number of species and genera in twenty-eight families of angiosperms. In a subsequent paper, chromosome numbers will be listed for members of many additional families. A discussion of the results presented in the two papers will be found at the end of the second paper.

McMASTER UNIVERSITY,
HAMILTON, ONTARIO, CANADA

LITERATURE CITED

- BOWDEN, WRAY M. 1940a. Diploidy, polyploidy, and winter hardiness relationships in the flowering plants. *Amer. Jour. Bot.* 27:357–371.
- . 1940b. The chromosome complement and its relationship to cold resistance in the higher plants. *Chronica Botanica* VI:123–125.
- GAISER, L. O. 1926. A list of chromosome numbers in angiosperms. *Genetica* 8:401–484.
- . 1930a. Chromosome numbers in angiosperms II. *Bibliographia Genetica* 6:171–466.
- . 1930b. Chromosome numbers in angiosperms III. *Genetica* 12:161–260.
- . 1933. Chromosome numbers in angiosperms IV. *Bibliographia Genetica* 10:105–250.
- TISCHLER, G. 1927. Pflanzliche Chromosomen-Zahlen. *Tabulae Biologicae* 4:1–83.
- . 1931. Pflanzliche Chromosomen-Zahlen. *Tabulae Biologicae* 7:109–226.
- . 1935. Pflanzliche Chromosomen-Zahlen. *Tabulae Biologicae* 11:281–304.
- . 1936. Pflanzliche Chromosomen-Zahlen. *Tabulae Biologicae* 12:57–115.
- . 1938. Pflanzliche Chromosomen-Zahlen IV. *Tabulae Biologicae* 16:162–218.

DESYNOPSIS IN THE COMMON WHEAT¹

H. W. Li, W. K. Pao, and C. H. Li²

IN 1939, attempts were made by the writers to find polyploids in the common wheat through the study of twin seedlings. One of the twins that was obtained in F_3 from a cross of the pure line 18–3874 and the variety Quality, exhibited unusual chromosomal behavior. It had in most of the cases about 14 bivalents and 14 univalents in the first meiotic metaphase in microsporogenesis instead of the usual 21 bivalents. This striking fact caused the writers to examine cytologically segregating progenies in F_3

¹ Received for publication December 5, 1944.

Published under a special ruling of the Editorial Board.

Journal series No. 4 of the Rice and Wheat Improvement Institute of the Szechuan Provincial Agricultural Improvement Institute, Chengtu, Szechuan, China.

² Head, Associate Agronomist of the Rice and Wheat Improvement Institute of Szechuan and Senior Agronomist of the Kansu Provincial Agricultural Improvement Institute, respectively.

of the same cross. It was found that most of the progenies were normal in respect to their chromosomal behavior, pollen viability and set of seed. Others contained plants which behaved in the same way as the twin mentioned but with varying percentage of pollen viability and seed set. Other plants showed almost no bivalents with a decidedly low percentage of viable pollen and seed, although these also were subject to variation. This cytological behavior indicated that either asynapsis or desynapsis was involved. Since that time much work has been done, the results of which are reported in this paper.

CYTOLOGICAL STUDIES.—In the course of this study, approximately two thousand plants were examined cytologically. In the normal plants, there were invariably 21 bivalents. In the abnormal plants, however, there were variations from 0 to as many as 21 normally paired bivalents. The reason

for the existence of such a great variation will be discussed later. When a cell is devoid of any bivalents, the univalents are scattered all over the cell in the late prophase (fig. 1) and metaphase stages, and in the anaphase they seem to pass to the poles at random. However, a few cells were found in which all the univalents were lined up on the equatorial plate quite regularly, though the orientation of their centromeres to the spindle was rather haphazard. These then separated at random to their respective poles. This orientation of the univalents on the equatorial plate was, however, a rare occurrence.

Whenever bivalents were present in a cell, they lined up in the equatorial plane, leaving the univalents widely scattered over the cell. After the bivalents commenced to divide, the univalents either came to the equatorial plate, or else they gathered about the pole nearest to their former position (fig. 2 and 4). In telophase, the univalents which lagged behind could be seen in the equatorial plane (fig. 5). Sometimes they split, and the halves either went to

opposite poles, or both were included in one daughter nucleus. The division of the cells with 21 bivalents, on the other hand, was as regular as in plants with normal synapsis.

In the second division, chromosome behavior was conditioned by the number of univalents that entered into the first division. The more univalents there were, the more irregular was the second division, this resulting in the presence of micronuclei and polyspory. For instance, plant 1705-2 had mostly univalents in nearly all the cells examined. The weighted mean of the bivalents was 1.8 and the resultant spore groups were quite irregular (table 1).

It can be seen from table 1 that only 10 of 77 sporocytes examined were seemingly normal with respect to regular microspore formation. However, even in these, the size of the nuclei varied greatly, and it is probable that they had an unbalanced chromosomal constitution which would lead to poor pollen viability. Ordinarily more than 90 per cent of the pollen grains in this type of plant aborted. Ma-



Fig. 1-10.—Fig. 1. Metaphase I, no bivalent.—Fig. 2. Metaphase I, 1 bivalent.—Fig. 3. Metaphase I, 4 bivalents.—Fig. 4. Metaphase I, 12 bivalents; notice the increase of chiasma frequency.—Fig. 5. Telophase I; notice lagging of univalents.—Fig. 6. Types of bivalents in desynaptic plants. Unequal pairs are indicated by arrows, and a chromosome of the reduced-contraction type is marked with an asterisk.—Fig. 7. Chromosomes at metaphase showing reduced contraction.—Fig. 8. Progeny of desynaptic plant showing 50 chromosomes, among which a tetravalent, and two trivalents.—Fig. 9. Multivalent chromosomes in the progeny of some desynaptic plants.—Fig. 10. Ring chromosome of F_1 , Quality X 18-3874.

TABLE 1. Frequency of polyspory and micronuclei in plant 1705-2.

Spores per sporocyte		4						5						6				7		8
Micronuclei	0	1	2	3	4	5	6	0	1	2	3	4	5	1	2	3	4	2	3	3
Frequency	10	16	18	12	7	0	2	0	1	1	2	0	2	0	1	1	1	1	1	1
Totals	65							6						3				2		1

crosporogenesis was not examined, but it is assumed that it was similar to microsporogenesis, since practically no seeds ordinarily were formed in this type of plant. Nevertheless, this again is subject to great variation, the reason for which will be discussed later.

Progress of desynapsis.—In examining the early prophase stages of the plants in which the number of bivalents at metaphase approached zero, it was found that the chromosomes definitely were synapsed and presented essentially the same appearance as in normal plants. Unpaired chromosomes were observed very rarely in the zygotene stage.

During pachytene, in some of the plants studied, the paired chromosomes seemed to fall apart and the number of double threads was roughly correlated with the amount of pairing in diplotene, diakinesis and metaphase. This is in close agreement with the behavior of asynaptic plants in *Zea* (Beadle, 1933). In other plants, however, the chromosomes were paired during pachytene, and remained so until early or mid-diakinesis when they appeared as single, unattached univalents, or a few chromosomes might be attached to each other for the most part in an end-to-end fashion. The falling apart of the paired chromosomes in this latter type of plant took place later than in the first type, probably during diplotene. This dissociation during the late prophase of chromosomes synapsed in the normal manner during the early prophase in mutants of various plant species, referred to by previous workers as *asynapsis*, is here described as *desynapsis*, a term which is believed to describe more accurately the synaptic behavior of the chromosomes in these plants.

Bivalent association in desynaptic plants.—Such bivalents as are found in the desynaptic plants have mostly terminal chiasmata or end associations (fig. 6). Interstitial chiasmata are rare. As the number of bivalents in the nucleus increased, the frequency

of chiasmata or end associations per bivalent apparently increased (compare fig. 2, 3 and 4). This is in agreement with the situation in desynaptic plants of *Zea* and other species. Attempts were not made, however, to measure the chiasma frequency, as different bivalents are found in different cells; in other words, counts can not be made on the same bivalents in all the cells studied.

Other chromosome behavior.—In some plants, all of the chromosomes in the cell appear to be slender and drawn out during metaphase and early anaphase (fig. 7). In these cells there may be many or few bivalents. The chromosomes of a majority of the desynaptic plants do not behave in this way and it was not determined whether this behavior is a chance variation or a genetically controlled phenomenon.

INHERITANCE OF THE DESYNAPTIC GENE.—The discovery of the desynaptic character in the F_3 of a cross involving the pure line 18-3874 and the variety Quality was made early in the growing season when cool weather prevailed. In attempting to identify additional desynaptic plants in the original F_3 population and also in F_4 progenies later in the season when higher temperatures prevailed it was found that the expression of the desynaptic character was extremely variable. When it was discovered that the expression of the character was markedly influenced by temperature, as will be described later, the original cross was repeated and under conditions of low temperature favorable for making the classifications the F_2 data given in table 2 were obtained.

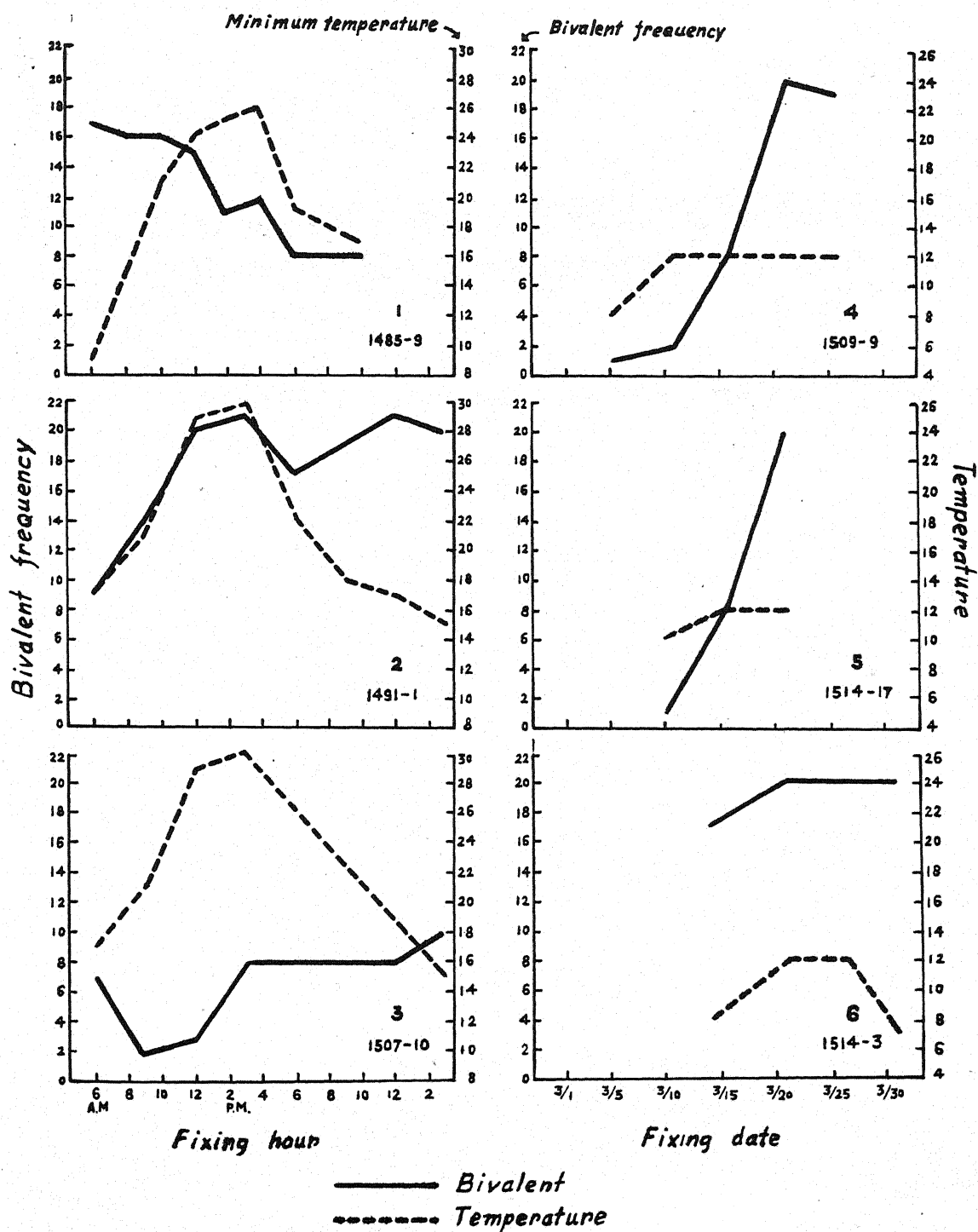
Of a total of 563 plants studied, 414 were normal and 149 desynaptic. Deviation from a monohybrid ratio was 8.25 ± 10.18 . The deviations in the separate lines used were not considered to be significant. The evidence points to a recessive desynaptic gene, designated *ds*. The following evidence suggests that the *ds* gene arose as a spontaneous mutation:

1. No abnormality was seen in the plants of the

TABLE 2. Segregation in F_2 of the cross normal \times desynaptic plants.

Line number	Cross combination		Observed		Expected		Diff. \pm s.e.	
			normal	desynaptic	normal	desynaptic		
1433	654-113	18-3874	95	39	100.50	33.50	5.50	5.01
1436-1	18-3874	654-20	42	14	42.00	14.00	0	3.24
1436-2	18-3874	654-20	11	5	12.00	4.00	1	1.73
1436-3	18-3874	654-20	73	23	72.00	24.00	1	4.24
1439-1	18-3874	654-177	35	9	33.00	11.00	2	2.88
1439-2	18-3874	654-177	52	25	57.75	19.25	5.75	3.80
1440-1	18-3874	654-177	106	34	105.00	35.00	1	5.12
Total			414	149	422.25	140.75	8.25 ± 10.18	

Text figures 1-6 showing variation of temperature and bivalent frequency



parental stocks that had been under observation for several years. Since wheat is normally self-pollinated, recessive characters would not be expected to

persist for many generations in the heterozygous condition but would be exhibited in the homozygous condition.

TABLE 3. *Variation of bivalent frequency in*

Plant number	Time of fixation		Temperature	Frequency distribution of cells										
				0	1	2	3	4	5	6	7	8	9	10
1485-9	3/31	6.30 a.m.	8.5 C	1	..
1485-9	3/31	8.30 a.m.	14.5 C	2
1485-9	3/31	10.30 a.m.	20.5 C	1
1485-9	3/31	12.30 p.m.	23.5 C	1	2	..
1485-9	3/31	2.30 p.m.	24.5 C	3	2	9	8	10	8
1485-9	3/31	4.30 p.m.	25.5 C	1	2
1485-9	3/31	6.30 p.m.	19.0 C	1	2	1	3	4	8	11	9	2	3	6
1485-9	3/31	8.30 p.m.	17.5 C	1	1	3	2	3	4	5	2	..	6	1
1485-9	3/31	10.30 p.m.	16.5 C	2	1	3	4	2	3	4	4	7	6	6
1491-1	4/3	6.00 a.m.	17.0 C	2	1	..	3	8	5	9	6	5	3	2
1491-1	4/3	9.00 a.m.	21.0 C	1	2
1491-1	4/3	12.00 a.m.	28.5 C
1491-1	4/3	3.00 p.m.	30.0 C
1491-1	4/3	6.00 p.m.	21.5 C	1	1	1
1491-1	4/3	9.00 p.m.	18.0 C
1491-1	4/3	12.00 p.m.	16.5 C
1491-1	4/4	3.00 a.m.	15.0 C
1507-10	4/3	6.00 a.m.	17.0 C	2	1	1	1	4	4	6	6	6	6	2
1507-10	4/3	9.00 a.m.	21.0 C	11	10	11	11	3	2	1	2	1
1507-10	4/3	12.00 a.m.	28.5 C	7	8	9	14	6	7	..	1
1507-10	4/3	3.00 p.m.	30.0 C	..	2	4	2	3	9	9	7	6	4	4
1507-10	4/4	3.00 a.m.	15.0 C	1	1	3	2	4	7

2. Repetitions of the original cross involving different individuals failed to repeat the same segregation. There appears to be no way of deciding how and when the mutation came about since the plants used in the original cross were lost.

INSTABILITY OF THE MANIFESTATION OF THE DESYNAPTIC GENE.—*Variation within a single day.*—The manifestation of this mutant gene appears to vary greatly from cell to cell and from plant to plant. The number of bivalents per cell ranges from zero to twenty-one. Studies were made therefore during 1942 and 1943 to find the factor or factors responsible for this variation. The first series of experiments was conducted in 1942. It was designed to determine the effect of temperature on the variability within a single day. Three desynaptic plants in F_6 were chosen at random and the spikes on the

tillers were fixed at intervals throughout the day. The temperature at the time of fixation was recorded. The results of this study are shown in table 3 and text-figures 1, 2, and 3.

It can be seen that the mean bivalent frequency of the first two plants used in this experiment, 1485-9 and 1491-1 varies more or less directly with the temperature. This was particularly true with plant 1491-1 with fixations from 6 a.m. to 6 p.m. But the two curves as seen in text-figure 2 split further apart from 6 p.m. on. The reason for this split is not clear. With plant 1485-9, the two curves ran almost parallel to each other from 2 p.m. on to 10 p.m. But the two curves diverged after that time. It seems, therefore, that when the temperature is low, the bivalent frequency is the lowest, the fullest manifestation of desynapsis. The bivalent frequency

TABLE 4. *Variation of bivalent*

Plant number	Date of fixation	Temperature			Frequency distribution							
		Max.	Min.	Average	0	1	2	3	4	5	6	7
1509-9	3/5	18.8	8.4	10.0	15	14	12	3	1	1
1509-9	3/11	18.0	12.0	14.2	9	14	13	11	2	1
1509-9	3/16	20.5	11.7	14.7	1	5	5	8	11	15
1509-9	3/21	16.5	12.2	13.2
1509-9	3/26	26.0	12.0	18.2	1	..
1514-17	3/10	19.5	10.0	14.7	41	23	14	..	1
1514-17	3/16	20.5	11.7	14.7	1	..	6	10	4	9
1514-17	3/21	16.5	12.2	13.2
1514-3	3/14	21.5	8.0	13.4
1514-3	3/21	16.5	12.2	13.2
1514-3	3/26	26.0	12.0	18.2
1514-3	3/31	25.5	7.2	15.9

material fixed at different times of the day.

with stated number of bivalents												Total number of cells counted	Mean of bivalents
11	12	13	14	15	16	17	18	19	20	21	22		
..	..	1	6	5	8	13	7	10	51	16.6
1	4	4	7	12	20	21	18	10	3	102	16.2
1	4	2	8	11	14	7	17	12	4	81	16.4
2	5	7	12	15	14	8	2	68	14.5
9	7	7	6	5	2	1	77	10.5
2	5	4	4	1	3	22	12.2
4	3	3	3	6	1	70	8.1
2	4	1	4	..	1	2	42	8.1
7	2	3	2	1	..	1	58	8.0
1	1	..	1	..	1	1	..	4	5	2	1	61	9.2
2	2	1	2	2	1	1	1	1	..	16	13.9
..	1	1	4	6	14	10	6	42	20.0
..	1	5	14	19	21	60	20.9
..	2	1	2	5	5	18	17.4
..	..	1	1	1	3	10	26	14	56	18.7
..	1	1	5	11	9	9	36	20.5
..	1	..	2	1	1	3	8	20.3
5	1	1	1	47	7.1
..	52	2.2
..	52	2.6
3	1	1	0	1	3	1	2	1	3	1	..	67	8.6
2	3	1	2	1	27	9.9

increases when the temperature rises and it approaches its highest level, yielding almost complete pairing, when the temperature gets above a certain level. This suggests, of course, that this desynapsis gene is stable in manifestation only during a period of relatively low temperature. It reverts to normal behavior at a higher temperature. On the other hand, plant 1507-10 had rather constant bivalent frequencies in spite of the temperature variation. Since it did not respond to variation in temperature as did the other two plants, it might be concluded that a modifying gene or genes might have been present for its control. This possibility will be investigated in later experiments.

Variation from day to day.—Another series of experiments was undertaken to determine the variation in bivalent frequency from day to day. In 1942,

frequency from day to day.

three desynaptic plants were chosen at random. Spikes of the tillers of these plants were fixed on various days, each fixation being made at 2 p.m. Temperature records on the day of fixation were obtained from a meteorological station about a mile from the experimental field. Unfortunately, only maximum, minimum and the average temperatures were available. These results are shown in table 4 and text-figures 4, 5, and 6. In the text-figures only the minimum temperature is shown.

It can be seen again that the bivalent frequency of the first two plants used in this experiment, 1509-9 and 1514-17, was rather low in the beginning of the experiment and rose quite high toward the end. Presumably, this variation is in accord with the variation in temperature judging from the temperature records, particularly the minimum temperatures.

of cells with stated number of bivalents														Total number of cells counted	Mean of bivalents
8	9	10	11	12	13	14	15	16	17	18	19	20	21		
..	46	1.2
..	50	1.7
10	10	9	8	6	1	2	91	7.8
..	3	3	18	16	7	47	19.5
..	1	2	1	5	8	9	10	37	18.8
..	79	0.7
6	2	3	..	1	5	3	2	3	55	8.3
..	6	45	51	102	20.4
..	1	3	3	10	18	16	20	33	13	9	1	127	16.8
..	11	48	58	117	20.4
..	1	3	7	30	21	62	20.1
..	4	3	7	20.4

TABLE 5. *Variation of bivalent*

Plant number	Date and time of fixation	Temperature C.			Frequency distribution							
		Max.	Min.	Average	0	1	2	3	4	5	6	7
1703-1	3/24	21.2	11.1	13.6	1	..	2	1
1703-1	4/8 8.30 a.m.	17.3	0.0	8.6	12	2	2	5	8	5	7	4
1703-1	4/8 10.10 a.m.	17.3	0.0	8.6	27	22	16	10	7	2	3	1
1703-1	4/8 1.30 p.m.	17.3	0.0	8.6	23	26	15	6	1	..
1703-1	4/10 7.30 a.m.	20.9	8.3	14.3	7	19	14	19	10	3	2	..
1704-1	3/24	21.2	11.1	13.6
1704-1	4/8 10.10 a.m.	17.3	0.0	8.6	9	16	24	20	16	3	2	1
1704-1	4/8 4.00 p.m.	17.3	0.0	8.6	..	10	20	18	11	22	13	3
1704-1	4/9 6.30 a.m.	15.9	7.8	11.4	2	1	1	..
1704-1	4/9 6.30 p.m.	15.9	7.8	11.4	14	4	15	12	3	1	1	..
1705-2	4/1	18.0	12.3	13.9	6	8	3	1
1705-2	4/8 10.10 a.m.	17.3	0.0	8.6	16	23	24	15	11	10	6	4
1705-2	4/8 1.30 p.m.	17.3	0.0	8.6	25	30	12	16	9	3	..	1
1705-2	4/8 8.30 p.m.	17.3	0.0	8.6	21	21	23	17	11	2	1	2
1705-2	4/9 12.00 a.m.	15.9	7.8	11.4	25	22	20	8	3	1
1705-2	4/10 7.30 a.m.	20.9	8.3	14.3	2	2	2	..	1	1
1705-2	4/11 7.30 a.m.	24.2	12.2	17.3	22	23	25	13	6
1705-2	4/11 1.00 p.m.	24.2	12.2	17.3	4	25	21	21	8	5	4	..
1705-2	4/12 10.45 a.m.	25.5	10.4	17.4	24	16	12	7	3	1
1705-2	4/12 6.30 p.m.	25.5	10.4	17.4	1	10	16	14	10	4	3	..
1695-2	4/8 10.10 a.m.	17.3	0.0	8.6	..	2	2	1	1	2	3	2
1695-2	4/8 11.40 a.m.	17.3	0.0	8.6	7	2	4	4	6	6	6	6
1695-2	4/8 4.00 p.m.	17.3	0.0	8.6	..	4	5	3	8	11	4	8
1695-2	4/9 6.30 a.m.	15.9	7.8	11.4	1	1	2	3	2	6	3	1
1695-2	4/9 6.30 p.m.	15.9	7.8	11.4	8	2	1	4	7	3	2	6
1695-2	4/10 2.45 p.m.	20.9	8.3	14.3	1	2	2	..	1	4	4	7
1695-2	4/11 1.00 p.m.	24.2	12.2	17.3	2	4
1695-2	4/12 10.45 a.m.	25.5	10.4	17.4	1	1	3	4	4	4	2	3

However, there are two difficulties that prevent one from drawing any definite conclusions:

1. As mentioned above, the desynapsis of the chromosomes takes place during pachytene to diplotene. It is not fully known as yet how long it takes the chromosomes to reach metaphase, at which stage the bivalent frequencies are observed.

2. Furthermore, the temperatures for the days during the experiment were not recorded. It is not safe, therefore, to draw any definite conclusion regarding how and when the reversion of desynapsis to normal was induced by the change in temperature. Yet, it appears safe to conclude that the variation in the bivalent frequencies in these two plants correlates well with the change in temperature in a general way.

On the other hand, plant 1514-3 continued to have a high mean bivalent frequency in spite of change in temperature. Here, modifying genes might be offered again in explanation.

In 1943, four desynaptic plants in the F₇ were selected at random for a replication of the experiment done in 1942. It can be noted that the season was cooler, so the experiment was started later than in the preceding year. On the 8th of April of this year there was a cold wave that greatly damaged the wheat crop. The results of the replicated experiment are shown in table 5.

Plants 1703-1 and 1704-1 can be considered together. The mean bivalent frequencies were high to start with at a relatively higher temperature. But as the cold wave set in, they dropped down and remained there even though the temperature was a little higher thereafter. It is of interest to note that during the cold spell, fixations were made more than once on certain dates, yet the mean bivalent frequencies remained at the lowest level. Plant 1705-2, on the other hand, had very low mean bivalent frequencies in spite of any change in temperature, and variation in temperature did not affect 1695-2 which showed on all fixation dates medium mean bivalent frequencies and a wide range of variability.

From the results of the above two experiments, several possible conclusions can be drawn:

1. Some of the plants seem to be affected greatly by a change in temperature. The higher the temperature, the higher the bivalent frequency and vice versa.

2. Below a certain minimum temperature, possibly around 10 degrees C., the mean bivalent frequency of this type of unstable desynaptic plant remains at a low level. Any rise of temperature automatically raises the mean bivalent frequency, until finally an almost complete pairing of the chromosomes in nearly all the cells is reached.

3. Some of the desynaptic plants are quite stable in spite of changes in temperature. Their mean biva-

frequency on different dates.

of cells with stated number of bivalents														Total number of cells counted	Mean of bivalents
8	9	10	11	12	13	14	15	16	17	18	19	20	21		
..	..	1	3	2	5	7	3	6	3	5	3	2	..	44	14.4
4	1	1	2	2	2	1	4	2	2	1	67	6.4
3	91	1.9
..	71	1.1
1	75	2.4
..	1	1	3	5	7	17	23	9	66	19.1
..	91	2.4
3	1	101	3.9
1	5	5.4
..	50	1.9
..	18	0.9
1	1	111	2.6
1	97	1.7
..	98	2.0
..	79	1.3
..	1	9	5.0
1	90	1.6
..	88	2.4
..	63	1.2
..	58	2.8
2	2	3	5	5	6	6	4	6	4	..	1	2	..	59	11.5
4	5	..	2	3	1	56	5.2
6	9	5	3	1	..	1	..	1	69	6.5
1	1	2	1	24	5.5
5	3	5	6	6	3	1	5	3	1	71	8.0
5	6	5	4	3	1	4	4	1	3	57	9.2
2	4	4	3	1	1	1	..	1	23	9.6
4	3	5	3	..	3	2	..	1	43	7.3

lent frequencies are either high, or very low, or else very variable with a mean which is around 10 bivalents per cell.

4. There were altogether ten desynaptic plants selected at random for these two experiments: six of these were unstable, two had constantly low, one had constantly high and one had extremely variable bivalent frequencies. Stability in manifestation of the desynaptic gene is possibly controlled by modifying genes and the constancy of the bivalent frequency is perhaps also controlled by another set of genes according to the segregating condition of these ten plants. However, as the number of plants used is none too great, it is not possible to draw final conclusions at this time.

VARIABILITY IN FERTILITY.—It follows from the instability of the manifestation of the desynaptic gene, that the seed set in the different desynaptic plants should vary greatly. Four F_6 plants were selected at random in 1942 and their tillers were marked in the order in which heading occurred. Unfortunately, no exact date was recorded. Presumably, there was some interval between the heading time for the various tillers, since the total interval between the heading of the first and last tillers is generally about two weeks. The number of seeds per spikelet was used as a criterion for the fertility of the plant. The results are shown in table 6.

From table 6, it can be seen that the fertility of plant 1510-7 rises from the first tiller onward and reaches its maximum on the last tiller. This maximum is comparable with that of the normal plant

TABLE 6. Relation between seed-set and sequence of heading of desynaptic plants.

Sequence of heading	Seeds per spikelet of different plants			
	1510-7	1491-1	1487-3	1482-6
1	0	.14	.84	1.08
2	.40	.17	1.43	1.73
3	.22	.04	.94	1.37
4	.21	.10	1.32	1.27
5	.92	.06	1.00	1.21
6	1.31	0.00	1.13	1.14
7	1.1172	1.38
8	1.47	...	1.00	1.40
935	.94	1.34

(for comparison, seeds per spikelet for F_1 of the cross Quality and 18-3874, recorded in 1940 range from 1.61 to 2.49). The air temperature in the spring of 1942, as mentioned earlier, rose gradually with the advancement of the season. This, presumably, influenced the bivalent frequency and consequently the fertility of the plant as expressed in terms of number of seeds per spikelet. This is in accord with the former finding.

TABLE 7. *Seasonal variation in the fertility of desynaptic plants.*

Date	Seeds per spikelet—class value										Total	Mean fertility	Mean temperature
	.1	.3	.5	.7	.9	1.1	1.3	1.5	1.7	1.9			
-3/10	23	6	4	1	2	1	1	..	38	0.33	12.5°C.
3/11-3/15	28	8	7	5	2	2	3	..	1	..	50	0.42	13.6°C.
3/16-3/20	18	10	17	6	5	5	..	1	62	0.47	15.9°C.
3/21-3/25	11	8	5	2	6	1	3	2	2	2	42	0.67	16.1°C.
3/26-	1	..	2	3	1.17	17.1°C.
Total	74	32	33	14	14	8	10	4	4	2	195	0.48

On the other hand, plant 1491-1 had a uniformly low seed set for all the tillers, plant 1487-3 had medium seed set, while plant 1482-6 had a rather high seed set for all tillers. These three plants were rather stable, therefore, and indifferent to change in temperature. Possibly, the amount of seed set may be correlated with the mean bivalent frequency. Should this be true, there would again be three distinct stable types of desynaptic plants which are not affected by the variation in temperature.

Variability in fertility of F_6 desynaptic plants can be seen again in the 1942 planting, as indicated by the number of seeds per spikelet in plants maturing at different times of the year. This is shown in table 7.

It can be seen from table 7 that the fertility of desynaptic plants varies tremendously. The majority of them are quite infertile, but some do have as high a fertility as normal plants. There seems to be a change from less fertile to more fertile plants as the season gets warmer.

The fertility of the desynaptic plants is directly compared with that of sister normal plants of the second generation in table 8.

TABLE 8. *Comparison of the fertility of normal and desynaptic plants of F_2 .*

Class value	.1	.3	.5	.7	.9	1.1	1.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	Total	Mean
Desynaptic	90	16	12	5	6	3	1	2	4	1	..	1	141	0.33
Normal	40	9	13	15	18	18	24	22	37	29	38	29	45	24	24	19	404	1.72
Total	130	25	25	20	24	21	25	24	41	30	38	30	45	24	24	19	545	1.36

The extreme variation in the fertility of normal plants in the second generation of the cross of the desynaptic plant and one of the original can be logically explained by the damage done by the late frost of 1943 as mentioned before. Nevertheless its mean is still much higher than that of the desynaptic plants, the difference being 1.39 seeds per spikelet. In the desynaptic plants, there is still a wide variation and the frequencies in each class are directly comparable with the results obtained in the previous year as shown in table 7 in spite of the frost damage. Unfortunately, individual desynaptic plants were not studied in detail, so that the mode of segregation of the modifying genes can not be analyzed from the data so presented.

PROGENY OF THE DESYNAPTIC PLANTS.—From the extreme irregularity of their meiotic behavior, it can be predicted that the chromosome number of the progeny of desynaptic plants would vary. This was found to be the case.

None of the 95 plants examined was found to have a chromosome number which was less than 42, indicating the non-viability of the $n-1$ gametes. The majority of the plants had the regular number of chromosomes, 42. Some, however, did have a higher number. Whether these n plus x gametes are transmitted through pollen or ovule, or both, has not been determined. The presence of the extra chromosomes should result in multivalent formation. Figure 8 shows the chromosome configurations in the plant with 50 chromosomes; a tetravalent, and two trivalents are present besides the regular bivalents. Figure 9 shows trivalents and tetravalent in the metaphase of other $2n$ plus x plants. No attempts have yet been made to study the progeny of extra-chromosome plants.

DISCUSSION.—Up to 1940 there has accumulated a voluminous literature on asynapsis in many genera, including *Zea* (Beadle, 1933), *Rumex* (Yamamoto,

TABLE 9. *Chromosome numbers of the progeny of the desynaptic plants.*

Chromosome number	Frequency
42	78
43	3
44	6
45	5
46	0
47	1
48	1
49	0
50	1
Total	95

1934), *Datura* (Bergner, Cartledge, and Blakeslee, 1934), *Crepis* (Richardson, 1935), *Pisum* (Koller, 1938), *Nicotiana* (Goodspeed and Avery, 1939), *Oenothera* (Catcheside, 1939), and *Allium* (Levan, 1940). In wheat, the only work on asynapsis that has been noted is that of Huskins and Hearne (1933). Asynapsis has been shown to be due to a simple Mendelian recessive in all cases where sufficient genetical work has been done, as in *Zea*, *Datura*, *Nicotiana*, and *Oenothera*. These genetically controlled failures of metaphase pairing seem to be characterized by (1) fairly complete zygotene and pachytene pairing of chromosomes, (2) gradual falling apart of the chromosome at diplotene, (3) reduced chiasma frequency and reduced contraction of the chromosomes, finally resulting in, (4) reduced bivalent frequency which is subjected to local variation leading to abnormal separation of the chromosomes and to irregular formation of gametes with variable fertility. This is true in the case of wheat in all the essentials. Since synapsis does take place in early prophase in all these genetically controlled cases of failure of metaphase pairing and the falling apart of the chromosomes takes place between pachytene and diplotene, it is considered that the term desynapsis describes these cases more accurately than the term asynapsis, which should be used only for those cases in which synapsis is absent during the first meiotic mitosis as originally defined by Randolph (1928). Thus, desynapsis is used throughout this paper.

It seems that the stability of the gene that causes the chromosomes to fall apart in the case of wheat depends on the environment to which the plants are subjected, particularly the variation in temperature. This gene is most effective when the temperature is the lowest, possibly below 10 degrees Centigrade. Only under conditions like this is the process of desynapsis complete. With the rise of temperature, this falling apart of the chromosome stops in some, till finally an optimum temperature is reached, then the genetically desynaptic plant will revert to complete pairing for all the chromosomes. This would mean, of course, that the higher temperatures overpower the effect of the desynaptic gene and keep the paired chromosomes from falling apart.

It seems that in wheat there are modifying genes that control the degree of manifestation of the desynaptic gene on the one hand, and constancy of the bivalent frequency is controlled by another set of modifying genes on the other. This complicates the situation. Unfortunately, the number of desynaptic plants used is not large enough to draw a final conclusion.

As expected from their different manifestation of the univalent frequency and their susceptibility to change of temperature, desynaptic plants fall into three classes in respect to fertility, namely, those with constantly low, those with constantly high fertility, and those which show variability as between different spikes of the same plant.

SUMMARY

1. Meiosis in desynaptic plants which arose following a varietal cross is described in detail.

2. Synapsis occurs in an apparently normal fashion in all of the plants. In some of the desynaptic plants the chromosomes fall apart in pachytene; in other plants they fall apart in diplotene.

3. Chiasmata in the desynaptic plants are mostly terminal at metaphase; rarely some are interstitial. Sometimes, the bivalents are unequal, suggesting the pairing of chromosomes not completely homologous.

4. Desynapsis is a simple Mendelian recessive character and its gene is designated by the symbol *ds*. It evidently arose as a spontaneous mutation after the original varietal cross.

5. There are stable and unstable types of desynapsis which are possibly controlled by modifying genes.

6. The mean bivalent frequency of the unstable type of desynaptic plants may show variation in relation to temperature.

7. The fertility of desynaptic plants is significantly lower than that of normal plants, but it shows considerable variation.

RICE AND WHEAT IMPROVEMENT INSTITUTE,
CHENG TU, SZECHUAN,
CHINA

LITERATURE CITED

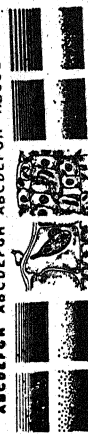
- BEADLE, G. W. 1930. Genetical and cytological studies of Mendelian asynapsis in *Zea mays*. Cornell Univ. Agric. Exper. Sta. Mem. 129.
- . 1933. Further studies of asynaptic maize. *Cytologia* 4: 269-287.
- BERGNER, A. D., J. L. CARTLEDGE, AND A. F. BLAKESLEE. 1934. Chromosome behavior due to a gene which prevents metaphase pairing in *Datura*. *Cytologia* 6: 19-37.
- CATCHESIDE, D. G. 1939. An asynaptic *Oenothera*. *New Phytologist* 38: 323-334.
- GOODSPEED, T. H., AND P. AVERY. 1939. Trisomic and other types in *Nicotiana sylvestris*. *Jour. Genet.* 38: 382-427.
- HUSKINS, C. L., AND E. M. HEARNE. 1933. Meiosis in asynaptic dwarf oats and wheat. *Jour. Royal Microsc. Soc.* 53: 109-117.
- KOLLER, P. C. 1939. Asynapsis in *Pisum sativum*. *Jour. Genet.* 36: 275-305.
- LEVAN, A. 1940. The cytology of *Allium amplexans* and the occurrence in nature of its asynapsis. *Hereditas* 26: 353-394.
- RANDOLPH, L. F. 1928. Chromosome numbers in *Zea Mays* L. Cornell Univ. Mem. 117: 1-44.
- RICHARDSON, M. M. 1935. Meiosis in *Crepis*. II. Failure of pairing in *Crepis capillaris* (L.) Wallr. *Jour. Genet.* 31: 101-117.
- YAMAMOTO, Y. 1934. Reifungsteilungen bei einer asynaptischen Pflanze von *Rumex acetosa* L. *Bot. Zool.* 2.

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

1234567890

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

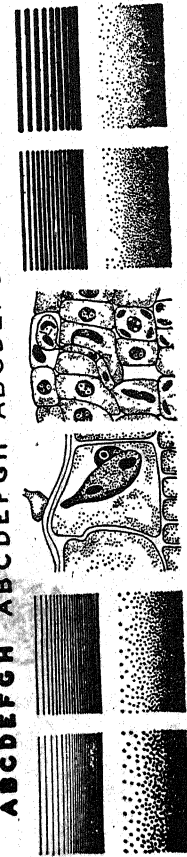


ABCDEF GHIJ KLMNOP QRSTUV WXYZ
1234567890

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

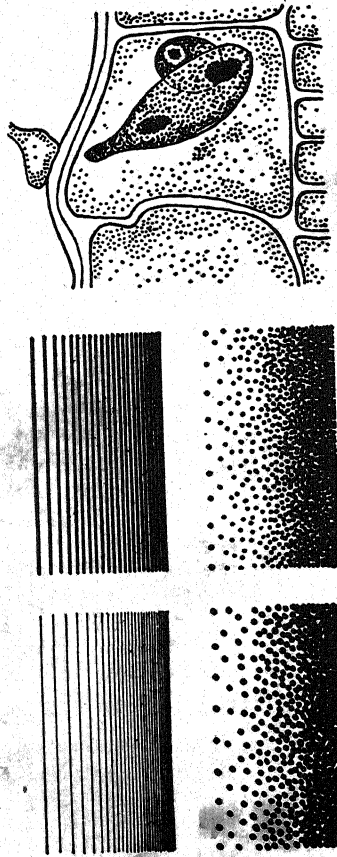
ABCDEF GHIJ KLMNOP QRSTUV WXYZ



ABCDEF GHIJ KLMNOP QRSTUV WXYZ
1234567890

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

ABCDEF GHIJ KLMNOP QRSTUV WXYZ



DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to $\frac{1}{4}$. Middle—Reduction to $\frac{1}{2}$. Bottom—Original size.

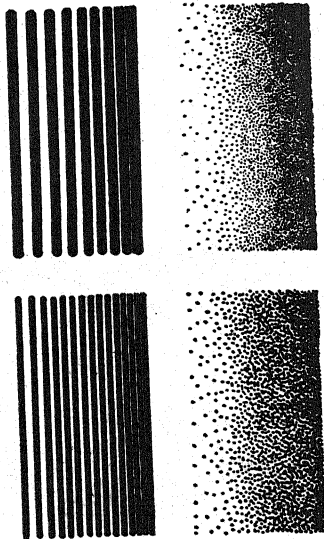
Reprinted by permission of the authors from: RIKER, A. J., and REGINA S. RIKER. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

ABCDEF GHIJ KLMNOP QRSTUV WXYZ

ABCDEF GHIJ KLMNOP QRSTUV WXYZ



STUDIES ON COLCHICINE-INDUCED AUTOTETRAPLOID BARLEY. I AND II. CYTOLOGICAL AND MORPHOLOGICAL OBSERVATIONS¹

Shao-lin Chen, Shu-min Shen, and P. S. Tang

THE OCCURRENCE of polyploid plants in nature has been known for a long time, and their artificial induction by the use of physical and chemical agents has been an accomplished fact since the beginning of this century (see anonymous: *The Experimental Production of Haploids and Polyploids*, 1936). With the discovery by Blakeslee and others that polyploid plants may be induced almost at will by the use of colchicine, the problem of polyploidy in plants has aroused renewed interest not only from the standpoint of the study of chromosome mechanisms and their significance in evolution, but also from the practical standpoint of plant breeding. The literature pertaining to the theoretical aspects of polyploidy in plants has been summarized by Müntzing (1936), Darlington (1937), Stebbins (1940), Blakeslee (1941), and others. As to the practical aspect of the problem, the situation is not yet wholly clear. While some consider that the artificial induction of polyploidy in plants may lead to many practical results in plant breeding and plant synthesis (Blakeslee, 1941; Randolph, 1941), others (Emsweller and Ruttle, 1941) are rather skeptical regarding this aspect of the problem. In any event, it may be said that up to the present, no results of major importance have been obtained in the field of plant breeding as a result of the induction of polyploidy by colchicine or other physical and chemical agents. This situation may be due to the fact that there has not been sufficient time since the discovery of the action of colchicine in 1937 to allow for the establishment of economically important polyploid plants by this means.

The present series of papers gives the results of the observations made on several strains of barley isolated from polyploid plants which were induced by the action of colchicine in our laboratory in the winter of 1939-1940 by Tang and Loo (1940), and which have been maintained to the present. The results of the observations made on their cytological and morphological characteristics are reported here.

CYTOLOGICAL OBSERVATIONS.—*Material and methods.*—Barley seeds of a pure 6-rowed strain obtained from the Division of Plant Pathology of this university were used in these experiments. The seeds were soaked in water over night from November 15 to 16, 1939 (by S. W. Loo). After soaking, the seeds were transferred to a solution of 0.05 per cent colchicine for 48 hours at the end of which they were planted in garden soil in wooden boxes

25 × 30 cm. and 10 cm. deep. Similar boxes containing untreated seeds were placed alongside as controls. In the earlier experiments, root tips of both the treated and untreated plants were fixed at the time of planting with Nawaschin's solution, stained with gentian violet, and their chromosome numbers were counted. This method was found to be too laborious, and in subsequent seasons pollen mother cells were examined by the use of McClintock's acetocarmine smear method instead of the paraffin section technique.

Results.—Out of 149 lines of $4n$ offspring examined, one line was found to produce both haploid and diploid gametes within the same anther. Seven lines reverted to diploids (fig. 1) in so far as their chromosome numbers were concerned but differed from ordinary diploids in possessing a lowered fertility. In a few lines, pollen mother cells with chromosome numbers ranging from 24 to 30 were observed (fig. 2). It is also interesting to note that in some lines it was impossible to count the chromosomes since they had lost their identity during metaphase and were fused together to form a mass of chromatin material (fig. 3). The pollen produced in these lines was, as might be expected, abortive. Most of the lines examined have been shown to possess 28 chromosomes, that is, the autotetraploid number, but this does not mean that these lines are all stable strains with constant chromosome numbers in their offspring. On the contrary, all kinds of irregularities have been observed in these lines: multivalents, including quadrivalents and trivalents of different shape have been found. Only a few of the 149 lines have shown regular chromosome division (fig. 4) in which 14 bivalents were formed (fig. 5, 6).

Owing to the formation of multivalents, segregation was naturally abnormal so that during anaphase unequal numbers of chromosomes pass to the two poles, resulting in the formation of abortive pollen and polysomic progenies (fig. 7). Microspores possessing 12 to 16 chromosomes were not uncommon, and laggards were also observed (fig. 8), but polyspory has not been noticed so far. The amount of sterility in the different lines was correlated with the formation of multivalents and other irregularities during meiosis. The fertility of a great majority of $4n$ plants was much lower than that of the diploids. Only in those lines in which 14 bivalents were formed in the metaphase was a degree of fertility comparable to that of the diploids found (fig. 9).

The $4n$ plants examined may be classified into the following groups: Group (1). Synapsis ex-

¹ Received for publication September 14, 1944.
Manuscript received through the Department of State, Washington, D. C., and published under a special ruling of the Editorial Board.

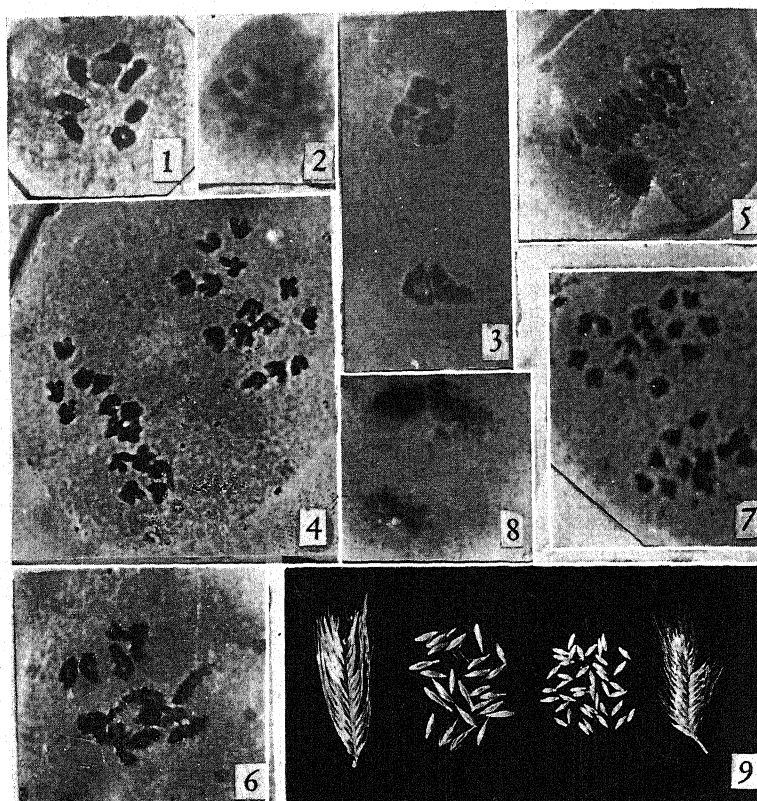


Fig. 1-9.—Fig. 1. Pollen mother cell of normal diploid plant; seven gemini are clearly shown during diakinesis.—Fig. 2. Pollen mother cell of an unstable tetraploid strain where variable chromosome number is found; 15 chromosomes at the second metaphase are shown in this figure.—Fig. 3. Pollen mother cell of an extremely abnormal strain where no chromosome identity can be recognized but a mass of chromatin material.—Fig. 4. Anaphase of a tetraploid strain showing normal segregation of chromosomes.—Fig. 5. Side view of the first metaphase of a tetraploid strain showing 14_{II} . Fig. 6. Pollen mother cell in diakinesis stage of a tetraploid strain showing 14 gemini.—Fig. 7. Anaphase of a tetraploid strain showing unequal segregation of chromosomes, with 15 to one side and 13 to the other.—Fig. 8. Anaphase of a tetraploid strain showing a laggard.—Fig. 9. A comparison of the spikes and seeds of tetraploids (left) and diploids (right).

tremely abnormal; the chromosomes could not be identified, being fused together into a mass of chromatin material. The pollen grains were all abortive, resulting in a high degree of sterility. Group (2). Both haploid and diploid gametes were formed within the same anther; offspring were all fertile, with type of head reverting to the diploid form. Practically no distinction could be made between the control and the offspring of this type except that more sterile spikelets were found in the latter. It is interesting to point out that since both haploid and diploid gametes were formed in the mother plants, there was every reason to expect the occurrence of diploid, triploid, as well as tetraploid seeds among their progenies, but we have failed to find polyploid seeds in their offspring so far. Group (3). Chromosome number was not constant, ranging from 24 to 30 . The progenies of this group were highly sterile. Polysomic plants were found in this group. Group (4). The plants in this group were all autotetraploids, and multivalents were formed during meiotic metaphase. The segregation of the chromosomes during anaphase was abnormal, re-

sulting in the formation of gametes with different chromosome numbers. Group (5). Autotetraploid plants with 14 bivalents formed in the meiotic metaphase. Offspring all showed the usual external features of tetraploids. Fertility of these plants, although lower than in the control diploids, was rather high in comparison with tetraploid plants of the other group. It is hoped that some stable tetraploid strains may be isolated from the offspring of this type.

MORPHOLOGICAL OBSERVATIONS.—As has been pointed out by various earlier workers, polyploid plants may be easily distinguished from the normal diploids in possessing the following characteristics: larger stomata, greener and broader leaves, heavier seeds, slower rate of growth and sturdier form, etc. The leaves of the tetraploid barley in our case were larger and greener than in the diploid plants, and the stomata were larger in size. The seeds of the $4n$ plants were heavier, ranging from 0.04 to 0.05 gm. per seed while those of the diploids weighed approximately 0.03 gm. The tetraploid plants germinated and headed more slowly than the diploids,

TABLE 1. *Test of significance of difference between means of some characters of agronomic interest in diploid and autotetraploid barley.*

Characters	$M_{4n} \pm S.D.$	n_{4n}	$M_{2n} \pm S.D.$	n_{2n}	d	σ_d	t	D.F.	1% level of t
Number of tillers.....	7.84 ± 4.23	31	7.47 ± 2.44	27	0.35	0.868	0.40	56	2.660
Length of stem (cm.)....	57.32 ± 8.75	31	87.03 ± 15.19	27	29.70	3.270	9.08 ^a	56	2.660
Diameter of stem (mm.)	6.37 ± 1.39	31	4.47 ± 1.04	27	1.90	0.318	5.97 ^a	56	2.660
Length of spike (cm.)...	4.57 ± 1.36	123	4.76 ± 1.02	166	0.18	0.146	1.23	287	2.576
Width of spike (cm.)....	1.46 ± 0.42	123	1.46 ± 0.19	166	0.003	0.042	0.07	287	2.576
Fertility (%)	43.67 ± 14.75	162	85.29 ± 5.42	62	41.62	5.680	7.33 ^a	222	2.576

^a Difference significant.

and hence matured later. When sown at the same time, the $4n$ seeds usually germinated two to three days later than the diploid seeds, and the plants headed two weeks later (about March 24 for the diploids and April 4 for the tetraploids). The tetraploids matured one week later than the diploids (about April 30 and 23, respectively).

Special attention has been paid to certain characteristics of agronomic importance in the tetraploids and diploids, and the data are statistically summarized in table 1.

From this table it is seen that no difference existed between the tetraploids and diploids in the number of tillers formed, though many of the tillers failed to produce fertile heads in some tetraploids. The stems of the tetraploids were thicker and stouter than those of the diploids, but shorter. From the average figures given in the table, no difference seems to have existed between the tetraploid and diploid plants, in size of the spikes. This does not, however, represent the true situation. As a matter of fact, the spikes of the tetraploid plants reached in some cases a length of 8 cm. and a width of 1.9 cm., dimensions much larger than those of the spikes of the diploid plants; on the other hand, many tetraploid spikes were sterile, and these were much smaller in size. By averaging the large, fertile spikes with the small, sterile spikes of the tetraploids, the differences between the tetraploid and diploid spikes disappear. Actually, if only the fertile spikes of the tetraploids are considered, the situation is quite different, and there appears little doubt that the spikes of the tetraploid plants, when fertile, were much larger than those of the diploids.

As pointed out above, autopolyploids are always partially sterile, that is, the ratio of percentage of seed set to total number of flowers is much higher in the diploids than in the tetraploids. In our case, the mean percentage of fertility in the tetraploids was only about 44 per cent, with a standard deviation of 14.75, while the mean fertility of the diploid plants was 85 per cent, and the range of variation was much smaller. That the fertility of the tetraploids was more variable and hence the tetraploid plants were less homogeneous than the diploids may be seen by a comparison of the standard deviations of the two series, and by a test for the significance of the difference.

$$d = 14.75 - 5.42 = 9.33.$$

$$\sigma_{\sigma_1 - \sigma_2} = \sqrt{\frac{(14.75)^2}{2 \times 162} + \frac{(5.42)^2}{2 \times 62}} = .954.$$

$$t = 9.33 / .954 = 9.78.$$

The value of t in this case turns out to be much larger than the 1 per cent value of t , hence the difference is significant, that is, the tetraploids were more variable and therefore less homogeneous than the diploids. From this more or less heterogeneous population it is quite likely that some fertile strains may be isolated which may be comparable with, if not higher than, the diploids in fertility.

DISCUSSION.—It may be seen from these cytological and morphological observations that the characteristics of polyploid barley conform to those of other polyploids obtained by other workers. Examination of the meiotic chromosomes in these autotetraploid plants revealed five different groups of plants from the standpoint of chromosome behavior. In one of these groups haploid and diploid gametes were found within the same anther. As a result of this phenomenon we might expect to find the occurrence of diploid, triploid and tetraploid seeds among the progenies, but actually only the diploid forms were found. It is possible that here certation played an important role, rendering the diploid pollen grains less effective than the haploid pollen so that only diploid progenies resulted in the offspring. Certation cannot be the sole cause of the total absence of polyploid offspring, however. If eggs containing $2n$ chromosome number are fertilized by haploid pollen, triploids should still occur. But in this case, two other factors should be considered: the ability of the diploid eggs to function during fertilization, and the probability of the formation of triploid progenies. Further observations on this matter may throw some light on this problem.

The tetraploid barley plants which we obtained, if fertile, possessed larger spikes and sturdier form than the diploids. Whether these plants may prove to be of economic value still remains to be seen.

SUMMARY

Autotetraploid barley plants have been induced in our laboratory by the use of colchicine and these plants have been maintained since 1939.

Cytological observations made on these tetraploid plants revealed various irregularities in chromosome configuration, but in a few lines regular bivalents were formed from which stable strains may be isolated in the future.

Morphological characters of agronomic interest are statistically treated and the findings agree with

those of the other workers on other polyploid plants.

The fertility of the $4n$ plants as a whole was lower than that of the diploids.

PHYSIOLOGICAL LABORATORY,
TSING HUA UNIVERSITY,
KUNMING, CHINA

LITERATURE CITED

- ANONYMOUS. 1936. The experimental production of haploids and polyploids. Imperial Bur. Pl. Genet.
BLAKESLEE, A. F. 1941. Effect of induced polyploidy in plants. *Amer. Nat.* 75:117-135.
DARLINGTON, C. D. 1937. Recent advances in cytology. Philadelphia, Blakiston's Son and Co.
EMSWELLER, S. L., AND M. L. RUTTLE. 1941. Induced polyploidy in floriculture. *Amer. Nat.* 75:310-326.
MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. *Hereditas* 21:263-378.
RANDOLPH, L. F. 1941. An evaluation of induced polyploidy as a method of breeding crop plants. *Amer. Nat.* 75:347-363.
STEBBINS, G. L., JR. 1940. The significance of polyploidy in plant evolution. *Amer. Nat.* 74:54-68.
TANG, P. S., AND S. W. LOO. 1940. Polyploidy in soybean, pea, wheat and rice, induced by colchicine treatment. *Science* 91:222.

GROWTH STIMULATION BY MANGANESE SULPHATE, INDOLE-3-ACETIC ACID, AND COLCHICINE IN THE SEED GERMINATION AND EARLY GROWTH OF SEVERAL CULTIVATED PLANTS ¹

Tsung-Lê Loo and Yü-Wei Tang

IN A previous paper, one of the writers (Loo, 1942) reported the effect of manganese sulphate, indole-3-acetic acid and colchicine on the germination and early growth of rice plants. In a concentration of 1 to 50 mg./liter, manganese sulphate increased the percentage of germination, the elongation of shoot and root especially, and also, the amount of dry substance. Seeds of rice in colchicine solution also germinated normally and rapidly, and the seedlings grew better than, or at least as well as, those in the control. On the contrary, the effect of indole-3-acetic acid was not very favorable. Rice seedlings grown in it showed a root which was thick and short with a tumor-like swelling at the tip. A low concentration of indole-3-acetic acid (1 mg./liter) stimulated the rate of germination a little, but a high concentration (50 mg./liter) was definitely growth-inhibiting. The length and the dry weight of the seedlings grown in this acid were also inferior to those grown in redistilled water and in solutions of manganese sulphate and colchicine. Thus the superiority of manganese sulphate over indole-3-acetic acid for the rice plant would appear to be established. However, since the concentrations used in the above-mentioned experiments were 1 to 50 mg./liter, it seemed desirable to confirm the facts in a wider range of concentrations and with materials other than rice plants. With this in mind, the writers conducted a series of experiments with mungo bean, maize, and cabbage as test materials, together with a study of the after-effect of soaking of the seeds in these solutions on the subsequent development of the embryo. The results of these experiments are described below.

¹ Received for publication September 28, 1944.

METHODS.—Seeds were soaked in tap water for one hour, sterilized with 3 percent formalin for ten minutes, and after washing twice with sterilized distilled water, were put into sterilized Petri dishes (9×15 cm.), each containing, as a rule, 113 grains (in the case of maize, each Petri dish containing 33 grains) for germination in 5 cc. of either redistilled water or aqueous solutions of manganese sulphate, indole-3-acetic acid or colchicine. All experiments were conducted in triplicate and every experiment was repeated three times. Manganese sulphate was a guaranteed reagent from E. Merck, Germany, and the indole-3-acetic acid and colchicine were obtained from the Mallinckrodt Chemical Works, U.S.A. The redistilled water used as the control and as the solvent in the solutions was specially prepared in this laboratory. Germination was carried out in an incubator in the dark.

After the frequency of germination was observed, 100 seedlings (in the case of maize, 50) out of the triplicated Petri dishes were selected from each test solution and transferred into a tall glass vessel (7×12 cm.) or a porcelain cup (8×9 cm.) containing 15 cc. of fresh test solution for further observation. These vessels were also placed in the incubator.

In the case of experiments dealing with the after-effect of the test solutions, the soaked and sterilized grains were immersed in 10 cc. of the test solutions of varied concentration each for a period of time. They were rinsed with redistilled water and were then transferred into the sterilized Petri dishes containing 10 cc. of redistilled water for germination. Early growth of the seedlings thus germinated was also observed by the method described above. At the

end of the experiment, the length and dry weight of the seedlings were determined and taken as criteria of the early growth.

EXPERIMENTAL RESULTS.—*The effect of manga-*

nese sulphate, indole-3-acetic acid and colchicine on seed germination and early growth.—The results of experiments with mungo bean, maize and cabbage are combined in table 1.

TABLE 1. *Effect of manganese sulphate (MnSO₄), indole-3-acetic acid (IAA) and colchicine (Col.) solutions upon the germination and early growth of mungo bean, maize, and cabbage. Control with redistilled water (H₂O). Root and shoot data are means of 100 seedlings (50 for maize). Germination counts made after the following periods (hrs.) in the order of conc. as listed: mungo, 19, 8, 8, 7; maize, 34, 35, 40, 32, cabbage, 18, 18, 19, 19. Measurement of length and dry weight made for mungo after 4 days for 10⁻⁵M., and 3 days for 10⁻⁶–10⁻⁸M.; for maize, after 4 days for 10⁻² and 10⁻³M., 5 days for 10⁻⁴ and 10⁻⁵M.; for cabbage, after 4 days for 10⁻³ and 10⁻⁶M. and 3 days for 10⁻⁴ and 10⁻⁵M.*

Plant	Conc. M.	Temp. C.	Solu. used	Percentage germination	Length (mm.) of root shoot		Dry weight (mg.) of root shoot		No. of roots
Mungo beans	10 ⁻⁵	25°	H ₂ O	69	103	52	130	858	...
			MnSO ₄	75	102	57	128	841	...
			Col.	75	99	52	128	797	...
			IAA	34	66	35	88	662	...
	10 ⁻⁶	25°	H ₂ O	42	43	79	109	858	...
			MnSO ₄	56	45	86	109	898	...
			Col.	55	38	78	97	762	...
			IAA	24	25	55	80	544	...
	10 ⁻⁷	24°	H ₂ O	47	37	71	101	850	...
			MnSO ₄	63	42	79	122	864	...
			Col.	64	42	81	122	892	...
			IAA	50	40	80	112	788	...
	10 ⁻⁸	24°	H ₂ O	37	42	62	127	739	...
			MnSO ₄	40	45	64	129	764	...
			Col.	41	47	67	127	761	...
			IAA	37	42	65	123	746	...
Maize	10 ⁻²	27°	H ₂ O	59	44	61	312	880	59
			MnSO ₄	47	11	44	123	712	47
			Col.	58	3	4	42	116	13
			IAA	0	0	0	0	0	0
	10 ⁻³	28°–29°	H ₂ O	77	50	61	378	833	321
			MnSO ₄	83	88	71	508	934	299
			Col.	76	10	15	202	598	223
			IAA	64	13	38	183	436	365
	10 ⁻⁴	25°	H ₂ O	87	55	60	357	1032	338
			MnSO ₄	90	72	58	404	1014	329
			Col.	85	51	58	427	1028	336
			IAA	78	36	58	384	983	372
	10 ⁻⁵	20°	H ₂ O	48	54	56	347	958	332
			MnSO ₄	49	55	60	392	961	341
			Col.	48	60	64	410	991	394
			IAA	44	34	50	315	829	377
Cabbage	10 ⁻³	20°–27°	H ₂ O	56	25	15	29	44	...
			MnSO ₄	51	22	10	26	36	...
			Col.	52	0	0	12	31	...
			IAA	1	0	0	0	0	...
	10 ⁻⁴	26°–28°	H ₂ O	64	13	6	16	34	...
			MnSO ₄	70	16	7	20	34	...
			Col.	72	13	5	13	38	...
			IAA	34	0	0	0	0	...
	10 ⁻⁵	26°–27°	H ₂ O	61	20	9	20	36	...
			MnSO ₄	76	16	8	19	35	...
			Col.	74	18	9	19	38	...
			IAA	77	20	9	21	33	...
	10 ⁻⁶	22°–23°	H ₂ O	52	29	15	21	48	...
			MnSO ₄	52	23	15	19	45	...
			Col.	58	26	16	16	33	...
			IAA	50	27	17	22	47	...

Mungo bean: Phaseolus Mungo var. *radiatus* Bak.—The concentration of the test solutions used in these experiments varied from 10^{-5} to 10^{-8} M. In solutions of manganese sulphate and colchicine in a concentration of 10^{-5} M., the seeds germinated rapidly showing somewhat higher percentage germination than the control at the end of nineteen hours. Seed germination in 10^{-5} M. indole-3-acetic acid solution on the other hand was only 50 percent of the control. The early growth of mungo bean seedlings was not accelerated in any of the test solutions. The concentration of 10^{-5} M. was apparently too high for mungo bean.

Manganese sulphate in concentrations of 10^{-6} M. and 10^{-7} M. fairly accelerated the rate of germination, the increase in percentage over the control being 14 and 16 respectively. The growth of seedlings in three subsequent days was also slightly superior to that of the control. Almost the same conclusions may be drawn for plants grown in colchicine, although in 10^{-6} M. colchicine the length and the dry weight of seedlings were only equal to, or a little less than, those of the control. The rate of germination and early growth of mungo bean was retarded by 10^{-6} M. indole-3-acetic acid, but a 10^{-7} M. solution of this acid accelerated germination and growth of the root. Perhaps 10^{-7} M. was the optimal concentration for mungo bean. Further dilution diminished the effect of these test solutions; percentage of germination, length and dry weight of the seedlings grown in these solutions became nearly equal to those of the control.

Maize: Zea Mays L.—The concentration of the test solutions used in these experiments was varied from 10^{-2} M. to 10^{-5} M. Manganese sulphate, indole-3-acetic acid and colchicine in such a high concentration as 10^{-2} M. exerted no good effect on germination and early growth of the maize plant, especially indole-3-acetic acid, in which no seed germinated during the experiment. Even manganese sulphate delayed the germination and the subsequent development to some extent. It is interesting to note that such a high concentration of colchicine, a toxic alkaloid, did not inhibit germination appreciably. The percentage of germination of seed soaked in this solution at the end of thirty-four hours was as great as that of the control, though the growth of the seedlings was inhibited. Manganese sulphate in a concentration of 10^{-3} M. had quite a beneficial effect on the early growth of the plant, although in this solution only an insignificant increase in percentage of germination occurred, the increase in growth was considerable, especially in the case of root growth, which showed an increase of 34 percent over the control. Seed germination in 10^{-3} M. colchicine solution was also fairly good, though the rate of growth was greatly delayed. Germination and subsequent growth rate of maize in 10^{-3} M. indole-3-acetic acid solution were low. Notwithstanding the poor growth, the number of roots per seedling was highest in this solution. This is in keeping with the observation of Marmer

(1937) on *Triticum* and Thimann and Lane (1938) on *Avena*.

The results of an experiment in which maize kernels were soaked and grown in 10^{-4} M. test solutions were similar in the main to those mentioned above, though the growth of the shoot was less striking in manganese sulphate solution.

Seed germination and subsequent development of maize plants in 10^{-5} M. manganese sulphate and colchicine solutions were slightly better than the control, but those in indole-3-acetic acid solution were inferior. In this case, both colchicine and indole-3-acetic acid increased the number of roots, but those grown in colchicine gave a different appearance from those grown in indole-3-acetic acid, the former being white and long and the latter, thick and short.

For maize, the optimum concentration of manganese sulphate seems to be 10^{-3} M.

Cabbage: Brassica campestris L. subsp. *chinensis* Makino.—Seed of cabbage seems to be very sensitive to manganese sulphate, colchicine and indole-3-acetic acid. In a concentration of 10^{-3} M., colchicine and indole-3-acetic acid were definitely detrimental to the early growth of cabbage seedlings. The growth of plants in manganese sulphate solution was also slightly inferior to that of the control. Seeds soaked in 10^{-3} M. manganese sulphate and colchicine germinated quite promptly, but those in indole-3-acetic acid solution of the same concentration did not. Germination and growth of cabbage was very good in 10^{-4} M. manganese sulphate, especially in the case of the root, which showed an increase in length and in dry weight of more than 20 percent over the control. Plants in 10^{-4} M. colchicine solution grew tolerably well. On the other hand, no growth of plants took place in indole-3-acetic acid solution of this concentration, though about half of the seed began to germinate. Seeds germinated readily and quickly in both manganese sulphate and colchicine solutions. When the concentration of the test solutions was diluted to 10^{-5} M., seed germination was accelerated equally by manganese sulphate, colchicine and indole-3-acetic acid, but the subsequent development of the seedlings was not benefited by these solutions. Further dilution of test solutions to 10^{-6} M. had no effect on the germination and the growth of cabbage. It is worthy of note that the growth of seedlings grown in colchicine and indole-3-acetic acid solutions was not improved by dilution.

The facts presented above lead to the conclusion that manganese sulphate in a concentration as high as 10^{-4} M. accelerates the germination and early growth of cabbage, but indole-3-acetic acid in the same and higher concentrations retards them. Low concentrations of indole-3-acetic acid (10^{-5} to 10^{-6} M.) seem to be ineffective. Within the concentration range used in these experiments with cabbage, colchicine exerts a beneficial effect on seed germination but it causes depression of growth in

high concentration, though it has no such effect in the low ones.

Thus seeds and seedlings of mungo bean, maize and cabbage cultured in manganese sulphate solution in a concentration as high as 10^{-3} M. germinate more readily and grow more quickly than in redistilled water. This beneficial effect of manganese sulphate does not diminish when the solution is diluted to 10^{-8} M. Seedlings grown in this medium are perfectly normal. On the contrary, germination and early growth of these test materials are more or less depressed by the presence of indole-3-acetic acid. Even in the most dilute concentration (10^{-8} M. in the case of mungo bean), the percentage of germination and the length and dry weight of the seedlings grown in this solution rarely exceeded those of the control. Colchicine in low concentration stimulates the germination and growth of these test plants, but a high concentration of colchicine is growth-inhibiting. The results of experiments with cabbage show that colchicine is particularly effective in seed germination.

The stimulating effect of manganese upon plant growth has long been recognized. Micheels and De Heen as early as 1906 reported that a colloidal solution of manganese increased the percentage of germination, the weight of the seedlings and the length of the root of wheat. An excellent review of the literature previous to 1936 concerning the effect of manganese was given by Brenchley (1936). Since then, others have reported stimulation of growth by manganese. Only those papers which have a bearing on the points discussed are cited below. Webster and Robertson (1937) found that permanganates as well as manganous salts produced marked increase in growth of *Opuntia*. They were able to prove that the effect produced by permanganates was not wholly manurial. Schopp and Arenz (1938) found that boron and manganese at a concentration of 0.1 mg./liter of solution or of sand brought an increase of 28.13 percent of shoot growth in water culture and 23.81 percent in sand. Lack of manganese expressed itself through a general suppression of growth and the appearance of a strong chlorosis. Tokuoka and Morooka (1939) found that for the growth of beet, 0.1 p.p.m. of manganese sulphate gave best results, but manganese in 20 p.p.m. was lethal. In a later paper, Tokuoka and Zyo (1940) reported the same stimulating effect of manganese on the growth of rice plants in pot experiments. Manganese up to a certain maximum amount always increased the length of leaves, the number of stems and the yield of the crop. They found that the maximum amount of manganese for the yield was 300 p.p.m. and for the length of leaves, 600 p.p.m. Thus it becomes evident that the optimal concentration of manganese for certain periods of growth differs according to the plant, and that some plants can endure a high concentration, as for example maize in our case.

As to the effect of indole-3-acetic acid on plant

growth, opinions are still not wholly in agreement. It has been widely recognized that indole-3-acetic acid causes inhibition in the elongation of roots (Kögl, Haagen-Smit, and Erxleben, 1934; Faber, 1936; Lane, 1936; Meesters, 1936; Thimann, 1936; Bouillenne and Bouillenne, 1938; Bonner and Koepfli, 1939). We also have numerous reports describing the retardation effect of this acid on the growth of other plant organs or of the plant as a whole (Solacolu and Constantinesco, 1936; Leonian and Lilly, 1937; Pearse, 1937; Marmer, 1937; Pratt, 1938; Kaiser and Albaum, 1939). On the other hand, there are evidences of growth promotion by indole-3-acetic acid in low concentrations (Amlong, 1936; Geiger-Huber and Burlet, 1936; Fiedler, 1936; Thimann and Lane, 1938; Eaton, 1940; and Gross, 1940). As far as the data available in this laboratory show, indole-3-acetic acid retarded seed germination and early growth of seedling in high concentration, and in low concentration it caused abnormal growth of the root, though it had a slightly beneficial effect on germination and growth. In no case did the increase in growth produced by this acid significantly exceed that by manganese.

Studies dealing with the physiological effect of colchicine are relatively few. Havas (1937) treated wheat seeds with 3-5 cc. of aqueous solutions of colchicine, or a combination of *Viscum album* preparations with colchicine, and found that colchicine exerted at the beginning of its application a definite stimulation effect on the rate of development of the roots and root hairs. This effect was followed in five to eight days by a very marked depression of the growth rate, and in another day their growth was completely arrested. The growth-inhibiting influence of colchicine on the shoots began even earlier. Paton and Nebel (1940) found that the elongation of excised maize roots was retarded by colchicine of concentrations 1, 2, 3, and 4×10^{-4} M. The concentration of this alkaloid used by these authors is evidently too high. Our data show that colchicine in moderate concentrations benefits the early development of plants.

AFTER-EFFECTS OF SEED TREATMENT.—*Oryza sativa* L.—Rice seeds were soaked in manganese sulphate, indole-3-acetic acid and colchicine solutions in a concentration of 100 mg./liter for 24 hours, with redistilled water as the control. The results are summarized in table 2. Almost all the seeds germinated at the end of 24 hours. There was no significant effect of the pretreatment on germination and early growth: however, those seeds treated with manganese sulphate showed a little greater percentage of germination and a greater growth of shoot; the growth of root was of the same order as that of the control. On the other hand, seeds treated with colchicine and indole-3-acetic acid showed less favorable growth than those in redistilled water and in manganese sulphate, especially in respect to the root.

Triticum vulgare, "Tsung-Yi 136."—Unfortunately, no further experiments with rice seeds were

TABLE 2. *Effect of seed-treatment with manganese sulphate, colchicine, and indole-3-acetic acid (100 mg./liter) on the germination and early growth of rice plants. Germination counts made after 24 hours. Root and shoot data, mean of 100 seedlings, taken after 5 days. Time of seed-treatment, 24 hours. Temp. 22°C.*

Solution used	Percentage germination	Length (mm.) of		Dry weight (mg.) of	
		root	shoot	root	shoot
Redistilled water	91	60	53	196	262
MnSO ₄	95	64	55	203	274
Colchicine	83	59	55	189	259
Indoleacetic acid	90	49	53	178	244

carried out owing to the unavailability of the material at that time. In subsequent experiments, only wheat seeds were used as test plants. A local variety of wheat "Tsung-Yi 136" was kindly supplied by the Meitan Branch Station of the Central Agri-

cultural Bureau. Test solutions in concentrations of 100 mg./liter, 150 mg./liter and 200 mg./liter were used simultaneously. Duration of treatment was varied from 12 hours to 48 hours. The data of these experiments are summarized in table 3.

TABLE 3. *Effect of seed-treatment with manganese sulphate (MnSO₄), indoleacetic acid (IAA), and colchicine (Col.) solution upon the germination and early growth of wheat plants, variety Tsung-Yi No. 136. Zero concentration means seeds were soaked in redistilled water. Germination counts made after the following periods (hrs.) in the order of time, top to bottom: MnSO₄, 9, 6, 12; IAA, 14, 14, 14; Col., 24, 24, 59. Root and shoot data taken after the following periods (days), in the order of time, top to bottom: MnSO₄, 5, 5, 7; IAA, 7, 8, 8; Col., 6, 7, 8. Root and shoot data, mean of 100 plants.*

Solu. used	Treatment			Percentage germination	Length (mm.) of		Dry weight (mg.) of	
	Time, hrs.	Temp., C.	Conc. mg./L.		root	shoot	root	shoot
MnSO ₄	12	26°-28°	0	53	75	51	225	295
			100	54	74	54	215	322
			150	67	72	55	213	324
			200	58	74	55	222	310
	24	20°-27°	0	31	65	55	246	349
			100	34	83	58	275	372
			150	23	82	60	263	363
			200	29	59	54	206	348
	48	24°	0	76	38	44	142	253
			100	87	40	41	137	262
			150	91	38	43	148	234
			200	87	44	41	144	245
IAA	12	20°	0	76	65	57	218	307
			100	57	75	45	250	281
			150	41	75	52	258	298
			200	25	77	54	276	288
	24	18°	0	70	74	51	206	279
			100	40	76	49	210	227
			150	30	72	49	213	262
			200	21	70	47	222	256
	48	20°-22°	0	90	80	61	248	325
			100	74	73	53	274	279
			150	66	71	51	271	253
			200	41	63	43	237	210
Col.	12	23°	0	45	71	65	259	395
			100	55	72	61	242	364
			150	51	65	58	244	348
			200	52	56	52	220	322
	24	23°	0	40	82	70	287	378
			100	37	78	68	261	361
			150	40	79	66	274	349
			200	51	73	65	250	341
	48	23°	0	53	74	72	259	387
			100	63	68	62	227	318
			150	57	63	60	228	327
			200	53	69	61	235	306

TREATMENTS WITH THE MANGANESE SULPHATE SOLUTIONS.—Treatment for 12 hours.—Seed germination of wheat was somewhat accelerated by pretreatment with manganese sulphate in any concentration. In this case, the most favorable concentration seemed to be 150 mg./liter. For early growth, the same conclusion holds good. Pretreatment of seed with manganese sulphate showed no beneficial effect on root growth; the length and dry weight of root were equal to, or even less than, those of the control. Apparently the duration of treatment was not long enough to exert any effect on early growth of roots.

Treatment for 24 hours.—Owing to the short period of germination (six hours after soaking), the effect of treatment was not noticeable. However, elongation of root and shoot and increase in dry weight were stimulated by the treatment, at least with moderate concentrations. High concentration (200 mg./liter) seemed to be unfavorable to root growth.

Treatment for 48 hours.—Soaking of seeds in redistilled water and in the test solutions for 48 hours retarded the subsequent development of the embryo, so that the effect of manganese sulphate was not so marked as in the cases of shorter treatment. However, the rate of germination was accelerated by manganese sulphate in every concentration. The root growth was also slightly increased by the pretreatment.

The results of the above experiments indicate that pretreatment of seed with manganese sulphate had beneficial effect on the germination and the subsequent growth of the seedlings, provided that the concentration is not too strong and the time of treatment not too long. Our results agree completely with those of Zlataroff (1934) who found that seeds of chickpea and peanut treated with 0.5 percent solution of manganese sulphate before sowing grew faster than the controls for 12 to 20 days.

TREATMENT WITH INDOLE-3-ACETIC ACID SOLUTIONS.—Treatment for 12 hours.—The rate of germination was reduced by the pretreatment and the degree of retardation in the rate of germination was proportional to the concentration of the test solution. At first, the elongation of root and shoot was considerably retarded, especially in the case of high concentration (200 mg./liter). For example, no root and only a few shoots were discernible in seeds treated with 200 mg./liter at 12 hours after they were put into Petri dishes. Noticeable swellings were present in the tip of the roots, as described in a previous paper (Loo, 1942). The swelling of roots was also observable in seedlings treated with this acid in a concentration of 150 mg./liter. But this abnormal growth feature disappeared 70 hours after the seeds were put into the Petri dishes. At the end of the experiment (seven days), the length and the dry weight of the root system were a little greater than those of the

control, though the growth of shoot was always retarded by the pretreatment.

Treatment for 24 hours.—With longer treatment, the after-effect became more marked, even in seeds treated with 100 mg./liter. No root was noticeable before 36 hours. In the case of seed treated with higher concentrations, the roots did not appear until 44 hours after they were set for germination. The swelling of roots and its disappearance after a certain time were also observed. In general, the shoots appeared earlier than the roots, though their elongation was retarded. Later in the experiment the rate of root growth was much accelerated in the case of seedlings treated with a moderate concentration of this acid, so that at the end of the experiment, length and dry weight became equal or in some cases even superior to those of the control.

Treatment for 48 hours.—The growth of both shoot and root was retarded by pretreatment with indole-3-acetic acid. In the case of seeds treated with 200 mg./liter, no root appeared within 60 hours. The swelling of roots was much more marked than that following the 24-hour treatment, and its degree was proportional to the concentration of the test solutions. From the fourth day on, however, the growth of the treated roots became suddenly accelerated. Moreover, the rate of acceleration was much greater in high concentrations than in 100 mg./liter. These findings agree completely with those by Thimann and Lane (1938) with *Avena*. What is more interesting was that the rate of growth of the shoot of the treated seeds began to decline at almost the same time that the root began to accelerate, the degree of retardation being also proportional to the concentration of the test solutions. Within the duration of the experiment, shoot growth was inferior to that of the control. The dry weight of the root was slightly increased by seed treatment with moderate concentrations of indole-3-acetic acid, but the length of root was reduced irrespective of the concentration of the test solution.

The pretreatment of seed with indole-3-acetic acid thus had no beneficial effect on germination and early growth of wheat plants. The rate of germination was retarded by the treatment in proportion to the concentration of this acid. The length and dry weight of the shoot were always inferior to those of the control. As to the growth of root, pretreatment of seed with indole-3-acetic acid resulted in retardation at first but increased the length and weight to some extent toward the end of the experiment. But with higher concentration and longer duration of treatment, the growth of the root was also retarded.

There have been many reports on the after-effect of treatment with indole-3-acetic acid upon seed or seedling. Thimann and Lane (1938) found that preliminary treatment of 24 to 48 hour-old plants with concentrated indole-3-acetic acid solutions or the soaking of seed in the same resulted in the

inhibition of root growth. But if after the treatment the plants were transferred to water the inhibited roots grew at a gradually increasing rate until their length actually exceeded that of the controls. Furthermore, they treated oat seedlings with this acid and planted them in sand in the greenhouse, and found that the growth of the shoots of the treated plants showed marked acceleration. Our results agree in general with those of Thimann and Lane except that soaking seed with a concentrated solution of indole-3-acetic acid generally caused inhibition of growth. According to Albaum, Kaiser and Eichel (1940), the growth of oat coleoptiles was benefited when grains were soaked for 24 hours at room temperature in solutions of glucose and indole-3-acetic acid (10 mg./liter); early growth was retarded but the final length was increased. They also found that the rate of respiration of the grains immediately after the soaking was inversely related to the final length of the coleoptile, and hence directly related to the velocity of growth. They considered that oxygen content of the soaking medium was a factor limiting the growth velocity and final growth. But from the fact that the acceleration of growth on transferring the seedlings to water had some relation to the concentration of indole-3-acetic acid, oxygen content of the soaking medium could not be considered an important factor in growth inhibition. The cause of early retardation of growth and respiration may, perhaps, be due to the direct effect of indole-3-acetic acid. More recently, Y. Hwang and Pearse (1940) found that treatment of seed for two hours with indole-3-acetic acid in concentrations varying from 2–200 p.p.m. had no effect on the growth of oat, but in the case of broad bean it showed a slight growth acceleration at low concentration and a definite retardation at high ones. It is evident from our results and a review of literature on the subject that soaking seed with indole-3-acetic acid generally results in the depression of germination rate and subsequent development of the embryos. The degree of depression is proportional to the concentrations used and the duration of the treatment. On transferring the treated seeds or seedlings to water, the depressing effect of indole-3-acetic acid decreases gradually and subsequently the rate of growth increases. There may be many possible causes of growth depression by indole-3-acetic acid: retardation of respiration as reported by Albaum, Kaiser and Eichel (1940) may be one of them, and the inhibition of mobilization of reserve materials and their transport may be another. The acceleration of growth after the treated plants were transferred to water may be the result of depletion of indole-3-acetic acid which had been absorbed by the seeds and the relatively large supply of reserve materials. If that be the case, how should one explain the superior root growth of the treated plants compared with that of the control? Since acceleration of root growth was always accompanied by the suppression of shoot growth, it is not unreasonable

to assume that the length and the dry weight of the root increase at the expense of the shoot.

TREATMENT WITH COLCHICINE SOLUTIONS.—*Treatment for 12 hours.*—Soaking of wheat grains for 12 hours with colchicine resulted in the acceleration of seed germination, but had no good effect on the early growth. The length and the dry weight of both shoot and root were slightly inferior to those of the control irrespective of the concentration of the test solution.

Treatments for 24 and 48 hours.—Prolongation of the time of treatment to 24 and 48 hours gave poorer results. The growth of both shoot and root was depressed. The germination percentage of seeds treated with 200 mg./liter for 24 hours and with 100 mg./liter to 150 mg./liter for 48 hours was slightly greater than that of the control, but in general soaking with colchicine for more than 24 hours had no significant effect.

The above results lead to the conclusion that within the concentration range and the duration of treatment used in these experiments, soaking seeds with colchicine slightly stimulates the rate of germination, but it has no good effect on the early growth of wheat seedlings.

DISCUSSION.—The above results show the superiority of manganese sulphate over indole-3-acetic acid and colchicine in stimulating the rate of germination and the growth of mungo bean, maize, cabbage, rice and wheat. The beneficial effect of manganese on plant growth, especially in small quantities, has been long recognized (for extensive literature, see Brenchley, 1936). Its essentiality in plant nutrition and growth has also been clearly demonstrated by Steinberg (1935) for fungi and by Stout and Arnon (1939) for higher plants. Yet the mechanism of its effect is still not understood. Y. W. Tang and Y. Yao of this laboratory (1942) have proved that manganese sulphate in agar-block induces curvature of oat and wheat coleoptiles in the same way as some growth-promoting materials do. Their findings throw some light on this problem. Although a growth-promoting material must induce *Avena* curvature when it is applied unilaterally to the decapitated oat coleoptiles, a substance inducing *Avena* curvature may not necessarily be a growth-promoting material in the real sense of the word. If manganese sulphate is a growth-promoting substance, its presence must have a beneficial effect on every stage of plant growth. The results of earlier work with rice and of the present investigation substantiate in part this statement. It must be pointed out that plants respond to manganese, indole-3-acetic acid and colchicine in quite different ways. From observations of growth features, it seems safe to conclude that: (1) the growth of seedlings in manganese sulphate solution or of seedlings pretreated with this solution is quite normal; (2) indole-3-acetic acid is toxic to plants in high concentrations and ineffective in low ones, its presence usually inducing bulbous hypertrophy of the root tips; (3) colchicine in

high concentration is also toxic; but it stimulates growth in relatively low concentration. So far as the early growth of root and shoot is concerned, there is no indication that colchicine induces anomalous growth.

How does manganese function in growth promotion? Why do indole-3-acetic acid and colchicine fail to promote growth? In the present studies, all experiments were conducted in the dark without supply of nutrients, and ended within ten days; the physiological processes that might have been involved were (1) the mobilization of food reserves, (2) the transport of decomposition products, (3) resynthesis of protein, cellulose and other cellular constituents and (4) respiration. Although Zlataroff (1934) suggested that the effect of manganese sulphate on the early growth of the plant may consist in the acceleration of enzyme activity during mobilization of reserve material, there is as yet no direct evidence to this effect. Recent works of Lundegårdh (1939), Burström (1939a, 1939b, 1939c) and Noack and Pirson (1939) are of interest in this connection. Lundegårdh regards manganese as a catalyst which plays the role which iron plays in animal respiration. According to Lundegårdh, nitrate reduction occurs only when manganese and oxygen are supplied and respiration is active. Burström, and Noack and Pirson conclude that the presence of manganese in culture media is necessary for the assimilation of nitrates by plants. These results suggest the possibility of the beneficial effect of manganese on the enzyme activity in plant metabolism. Further investigations along this line are desirable.

Recently, numerous data concerning the effect of indole-3-acetic acid on the metabolic activities of plants have been accumulated (Mitchell and Martin, 1937; Mitchell and Hamner, 1938; Alexander, 1938; Stuart, 1938; Mitchell and Stuart, 1939; Mitchell, 1940; Mitchell, Kraus and Whitehead, 1940; Smith, Nash and Davis, 1940). This is not the place to enter into details. It is enough to say that up to the present, the beneficial effect of indole-3-acetic acid on plant metabolism is doubtful. As to the relation between colchicine and plant metabolism, Paton and Nebel's results (1940) are interesting. According to these authors, colchicine in a concentration of 10^{-4} M. lowers the rate of

respiration and dipeptidase activity of excised maize roots. Whether the ineffectiveness of indole-3-acetic acid and the harmful effect of colchicine on metabolic activities are the causes of their inferiority to manganese is a problem which remains to be solved. Experiments along this line are in progress in this laboratory.

SUMMARY

Manganese sulphate in a wide range of concentration accelerates the rate of seed germination and growth. In the case of maize plants, a considerable increase in length and in dry weight was secured in the presence of 10^{-3} M. and 10^{-4} M. manganese sulphate. The growth habit of seedlings in these solutions was perfectly normal. High concentration of indole-3-acetic acid inhibits the rate of germination and growth. Seed germination and growth in low concentration of indole-3-acetic acid were either as good as, or slightly better than those of the control. This acid stimulates the production of adventitious roots, but the roots are thick and short with prominent swellings at their tips. Colchicine in appropriate concentrations stimulates seed germination and causes slight increase of growth. High concentration of colchicine is growth-inhibiting. Roots grown in this solution appear quite normal, however.

Pretreatment of seed with manganese sulphate in suitable concentration has a favorable after-effect on the germination and growth of plants, provided that the time of treatment is not longer than 24 hours. Soaking of seed for more than 24 hours is unfavorable for subsequent development even in redistilled water. Soaking of seed with indole-3-acetic acid causes a depression of germination rate, but on transferring the seeds to water, elongation of the root is accelerated at the expense of the shoot. Treatment for longer than 24 hours retards the elongation of root and shoot. Swelling of root tips was also observed in this case. The after-effect of seed-soaking with colchicine on seed germination is fairly good, but the subsequent development of the treated seed is not favorable.

LABORATORY OF PLANT PHYSIOLOGY,
BIOLOGICAL INSTITUTE,
NATIONAL UNIVERSITY OF CHEKIANG,
MEITAN, CHINA

LITERATURE CITED

- ALBAUM, H. G., S. KAISER, AND B. EICHEL. 1940. Coleoptile growth after preliminary soaking of oat grains in various solutions. *Amer. Jour. Bot.* 27: 619-623.
- ALEXANDER, T. R. 1938. Carbohydrates of bean plants after treatment with indole-3-acetic acid. *Plant Physiol.* 13: 845-858.
- AMLONG, H. U. 1936. Der Einfluss der Wuchsstoffe auf die Wanddehnbarkeit der *Vicia Faba*-Wurzel. *Ber. Deutsch. Bot. Ges.* 54: 271-275.
- BONNER, J., AND J. B. KOEPFEL. 1939. The inhibition of root growth by auxins. *Amer. Jour. Bot.* 26: 557-566.
- BOUILLENNE, R., AND M. BOUILLENNE. 1938. Contribution à l'étude des facteurs de la neoformation et de la croissance des racines. *Bull. Soc. Roy. de Bot. de Belgique* 71: 43-67.
- BRENCHLEY, W. E. 1936. The essential nature of certain minor elements for plant growth. *Bot. Rev.* 2: 173-196.
- BURSTRÖM, H. 1939a. The catalysis of heavy metals of the assimilation of nitrate. *Planta* 29: 292-305.
- . 1939b. Effect of heavy metals on the nitrate assimilation of wheat roots. *Kgl. Landbruksakad. Tid.* 78: 35-52.
- . 1939c. The role of manganese in the assimilation of nitrate. *Planta* 30: 129-150.

- EATON, FRANK M. 1940. Interrelations in the effects of boron and indole-3-acetic acid on plant growth. *Bot. Gaz.* 101: 700-705.
- FABER, E. R. 1936. Wuchsstoffversuch an Keimwurzeln. *Jahrb. Wiss. Bot.* 83: 439-469.
- FIEDLER, H. 1936. Kulturen isolierter Wurzelspitzen. *Zeitschr. f. Bot.* 30: 385-436.
- GEIGER-HUBER, W., AND E. BURLET. 1936. Über der hormonaler Einfluss der β -Indolylessigsäure auf das Wachstum isolierter Wurzeln in keimfreier Organkultur. *Jahrb. Wiss. Bot.* 84: 233-253.
- GROSS, W. W. 1940. The response of shoot of mungo bean seedlings growing in solution of 3-indole-acetic acid. *Amer. Jour. Bot.* 27: 371-376.
- HAVAS, L. 1937. Effects of colchicine and *Viscum album* preparations upon germination of seeds and growth of seedlings. *Nature* 139: 371-372.
- HWANG, Y., AND H. L. PEARSE. 1940. The response of seeds and seedlings to treatment with indole-acetic acid. *Ann. Bot.* 4: 32-37.
- KAISER, S., AND H. G. ALBAUM. 1939. Early root and shoot growth in two varieties of *Avena sativa* in relation to growth substances. *Amer. Jour. Bot.* 26: 749-754.
- KÖGL, F., A. J. HAAGEN-SMIT, AND H. ERXLEBEN. 1934. Über den Einfluss der Auxine auf das Wurzelwachstum und über die chemische Natur des Auxins der Graskoleoptilen. *Zeitschr. Physiol. Chem.* 228: 104-112.
- LANE, K. H. 1936. The inhibition of roots by growth hormone. *Amer. Jour. Bot.* 23: 532-535.
- LEONIAN, L. H., AND V. G. LILLY. 1937. Is heteroauxin a growth-promoting substance? *Amer. Jour. Bot.* 24: 135-139.
- LOO, T. L. 1942. Growth stimulation by manganese, indole-3-acetic acid, and colchicine in the seed germination and early growth of rice plant, *Oryza sativa* L. *Sci. Rec.* 1: 229-237.
- LUNDEGARDH, H. 1939. Manganese as a catalyst of cell respiration. *Planta* 29: 419-426.
- MARMER, D. R. 1937. Growth of wheat seedlings in solutions containing chemical growth substances. *Amer. Jour. Bot.* 24: 139-145.
- MEESTERS, A. 1936. The influence of heteroauxin on the growth of root hairs and roots of *Agrostemma Githago* L. *Proc. K. Akad. Wetenschap. Amsterdam* 39: 91-97.
- MICHEELS, H., AND P. DE HEEN. 1906. The stimulating action of manganese on germination. *Acad. Roy. Belgique Bull. Cl. Sci. No. 5: 288-289.*
- MITCHELL, J. W. 1940. Effect of naphthalene acetic acid and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plants. *Bot. Gaz.* 101: 688-699.
- , AND C. L. HAMNER. 1938. Stimulating effect of β -indole-acetic acid on synthesis of solid matter by bean plants. *Bot. Gaz.* 99: 569-583.
- MITCHELL, J. W., E. J. KRAUS, AND M. R. WHITEHEAD. 1940. Starch hydrolysis in bean leaves following spraying with Alpha naphthalene acetic acid emulsion. *Bot. Gaz.* 102: 97-104.
- , AND W. E. MARTIN. 1937. Effect of indole acetic acid on growth and chemical composition of etiolated bean plants. *Bot. Gaz.* 99: 171-183.
- , AND N. W. STUART. 1939. Growth and metabolism of bean cuttings subsequent to rooting with indole acetic acid. *Bot. Gaz.* 100: 627-650.
- NOACK, K., AND A. PIRSON. 1939. Effect of iron and manganese on nitrogen assimilation of *Chlorella*. *Ber. Deutsch. Bot. Ges.* 57: 422-452.
- PATON, R. L., AND B. R. NEBEL. 1940. Preliminary observations on physiological and cytological effects of certain hydrocarbons on plant tissues. *Amer. Jour. Bot.* 27: 609-613.
- PEARSE, H. L. 1937. Effect of heteroauxin on the growth of broad bean plants in water culture. *Nature* 140: 26.
- PRATT, R. 1938. Influence of indole-3-acetic acid on the respiration and growth of intact wheat seedlings. *Amer. Jour. Bot.* 25: 389-392.
- SCHROPP, W., AND B. ARENZ. 1938. Effect of boron and manganese on the growth of the maize plant. *Phytopath.* 11: 588-606.
- SMITH, O., L. B. NASH, AND G. E. DAVIS. 1940. Chemical and histological responses of bean plants grown at different levels of nutrition to indole acetic acid. *Bot. Gaz.* 102: 206-216.
- SOLACOLU, T., AND D. G. CONSTANTINESCO. 1936. Action de l'acid β -indolyl acetique sur la developpement des plantules. *Compt. Rend. Acad. Sci., Paris* 203: 437-440.
- STEINBERG, R. A. 1935. The nutritional requirement of the fungus, *Aspergillus niger*. *Bull. Torrey Bot. Club* 62: 81-95.
- STOUT, P. R., AND D. I. ARNON. 1939. Experimental method for the study of the role of copper, manganese and zinc in the nutrition of higher plants. *Amer. Jour. Bot.* 26: 144-148.
- STUART, N. W. 1938. Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indole-3-acetic acid. *Bot. Gaz.* 100: 298-311.
- THIMANN, K. V. 1936. Auxins and the growth of roots. *Amer. Jour. Bot.* 23: 561-569.
- , AND R. H. LANE. 1938. After-effects of the treatment of seed with auxin. *Amer. Jour. Bot.* 25: 535-543.
- TANG, Y. W., AND Y. YAO. 1942. Induction of *Avena* curvature by manganese. *Sci. Rec.* 1: 223-229.
- TOKUOKA, M., AND H. MOROOKA. 1939. The influence of micro-elements on the growth of herbage plants. III. *Beta vulgaris* L. *Jour. Sci. Soil & Manure Japan* 13: 489-494.
- , AND S. ZYO. 1940. The effect of manganese on the growth of rice plants. *Jour. Sci. Soil & Manure Japan* 14, 335-344.
- WEBSTER, M. E., AND IVAN M. ROBERTSON. 1937. Permanganates and plant growth. *Nature* 139: 71.
- ZLATAROFF, A. 1934. Nouvelles contributions experimentales pour l'explication de l'influence des stimulants chimiques sur la croissance des semences des plantes. *Bull. Soc. Chem. Biol.* 16: 1720-1729.

GENETICS OF GLOMERELLA. II. FERTILIZATION BETWEEN STRAINS¹

C. W. Edgerton, S. J. P. Chilton, and G. B. Lucas

FROM STUDIES which have been made on certain fungi belonging to the genus *Glomerella*, the evidence supports the conclusion that there is a close association between two distinct strains or strain types which have been designated as plus and minus. The plus strain is the one ordinarily picked up and cultured. When ascospores are isolated from this strain, cultures of both the plus and minus strains are readily obtained.

It is also known that when the plus and minus strains are grown in the same plate, a ridge of perithecia forms very rapidly on the line where they come together. The perithecia develop more rapidly in this ridge than they do in the small glomerate masses which are produced by a plus culture growing by itself. When the ascospores from a ridge are cultured, it has been found that ordinarily the asci contain either four plus and four minus spores or else eight minus spores. As the same segregation occurs in the plus strain alone, it has not been possible to prove definitely that the ridge of perithecia on the line of contact follows a fertilization between the two strains as was suggested in an early paper (Edgerton, 1914), or is merely due to some stimulus.

The object of this paper is to present evidence to show that a fertilization does take place between the plus and minus strains. A culture of the plus type from *Ipomoea* has provided the way for obtaining such evidence. As reported in a previous paper (Lucas, Chilton, and Edgerton, 1944), from this culture and its plus and minus progeny several variants were obtained which were definitely distinct from the plus and minus strains usually isolated from ascospores. Certain of these when grown in plates with other strains produced perithecial ridges on lines of contact. From these ridges it was again possible to isolate ascospores and identify the cultures developing from them.

Among the variants obtained from the *Ipomoea* culture were two strains which were of great aid in the present investigations. These were a strain definitely of the plus type but differing in certain respects from the original better-known one and a culture of the minus type distinct from the original. These two variants and the common plus and minus strains have been grown in plates in all possible combinations.

The term *type* is introduced in this paper in order to group strains which differ in some respects but in general are similar. There are strains of the plus type which are unlike in certain characteristics, and similarly there are different strains of the minus type. For the present no other types are being considered.

DESCRIPTIONS OF CULTURES USED.—Cultures of the ordinary plus and minus strains and of the two

variants were obtained from ascospores of the original plus strain of the *Glomerella* from *Ipomoea*, from its progeny, or from ridges of perithecia between different strains. The important characteristics are briefly listed. For this paper, these four strains are designated as plus A, plus B, minus A, and minus B. It must be understood that these symbols refer only to the cultures used.

Plus A.—From the original plus culture and similar to it in all ways. Perithecia produced in scattered glomerate masses. Perithecia full of well-developed asci and ascospores. Asci when analyzed by culturing individual ascospores produce *ordinarily either four cultures of the plus type (plus A) and four of the minus type (minus A), or else eight of the minus (minus A)*. Conidia not observed.

Plus B.—Macroscopically indistinguishable from the plus A strain. Perithecia produced in scattered glomerate masses. Asci numerous and well developed. Asci, however, when analyzed by culturing individual ascospores, produce *ordinarily eight cultures of the plus type (plus B) and apparently none of the minus type*. (Evidence for this is presented in a later paragraph.) Conidia not observed. This strain originally came from a single ascospore from the line of contact between a minus and a conidial strain.

Minus A.—The common minus strain, similar to the minus strains described in previous publications. Originally from an ascospore from the plus A strain. Produces numerous perithecia, singly or in groups of two or three scattered over the surface of the agar. *Perithecia ordinarily sterile, only an occasional one producing a few asci*. Ascospores when isolated produce colonies of the minus A strain.

Minus B.—This strain designated in a previous publication as *fertile minus*. Originally from an ascospore from a plus A strain. Produces numerous perithecia singly or in groups of two or three scattered over the surface of the agar. *Most of the perithecia filled with well-developed asci and ascospores*. Ascospores when isolated produce colonies of the minus B strain.

STABILITY OF THE PLUS B STRAIN.—The plus B strain was first recognized when a culture of the plus type was obtained which produced cultures only of the plus type when subcultures were made from ascospores. This seemed unusual as both plus and minus types had previously been obtained from ascospores from all plus strains studied.

To determine whether this culture was stable and would not break up into plus and minus strains, it was tested from time to time during a period of two and a half years. Numerous isolates were obtained from single ascospores, from perithecial clumps, from the original culture and from subcultures which had developed from ascospores.

¹ Received for publication October 25, 1944.

TABLE 1. Identity of cultures from ascospores of plus B strain (culture 5-16 and its ascospore progeny) from 58 perithecial clumps.

Culture	Number of perithecial clumps	Plus type	Number of cultures		Total
			Conidial strains	Sterile strains	
By single ascospore					
5-16	22	325	4	19	348
5-16-170	17	176	2	13	191
5-16-170-2	3	103	0	0	103
5-16-A-1	8	216	0	0	216
5-16-I-1	2	53	0	0	53
5-16-A-1-C-2	1	56	0	0	56
5-16-A-1-D-5	1	35	0	0	35
By dilution					
5-16	4	592	0	0	592
Total	58	1556	6	32	1594

Most of these isolates were obtained by picking up single ascospores though some were from dilution cultures made with ascospores. As shown in table 1, cultures were obtained from 1594 ascospores. Of these, 1556 produced cultures of the plus type.

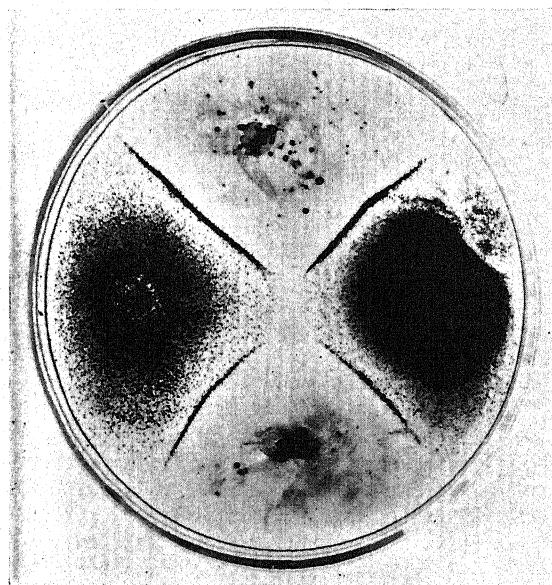


Fig. 1. Strains Plus A, Plus B, Minus A and Minus B, showing ridges of perithecia between plus and minus strains.

The other 38 cultures produced only conidia or sterile mycelium. No cultures of the minus type developed from any of these ascospores. It is believed that the evidence is sufficient to show that this strain does not produce the minus strain from ascospores.

CROSSING TESTS.—The two strains of the plus and the two of the minus types were grown in all possible combinations in petri dishes (fig. 1). Ridges of well-developed perithecia formed quickly on lines of contact when the following were grown together:

(1) plus A and minus A; (2) plus A and minus B; (3) plus B and minus A; (4) plus B and minus B. The asci in the perithecia in these ridges were analyzed by picking up the individual ascospores from a number of asci and growing them on oatmeal agar. The identity of the cultures that developed was determined. While it was not always possible to obtain cultures from all eight ascospores in an ascus, it was found that the constitution of an ascus could ordinarily be determined if cultures from five ascospores were obtained. As the asci in a perithecium are usually alike, having the same distribution of strains, asci were usually selected from a number of perithecia.

Plus A and minus A.—This is the well-known combination described in previous papers. It was known that the asci in the ridge of perithecia between these strains have either four ascospores of the plus and four of the minus strains or else eight of the minus. To make certain that the cultures being used acted similarly to those studied previously, the asci from a few perithecia were analyzed. Of eleven asci from the ridge of perithecia between these strains, five had four spores of the plus A strain and four of the minus A, and six had eight spores of the minus A. This was in agreement with previous work and no further tests seemed necessary.

Plus A and minus B.—Two different cultures of each strain were used in the crossing tests. A ridge of perithecia formed quickly on the line of contact when cultures of the plus A and minus B strains were allowed to grow together. The perithecia were well-developed and filled with asci containing normal ascospores.

The ascospores from 42 asci from the ridges were picked up and cultured. As shown in table 2, of these asci 22 apparently had four spores of the plus type and four of the minus B strain, while 19 had eight spores of the minus B strain. One had a 6:2 segregation.

Cultures of the plus type obtained from a few of the asci with the 4:4 segregation produced peri-

TABLE 2. *Asci in segregation groups from crosses between plus A and minus B strains.*

Cross number	Parents Plus A \times minus B	Number of asci in each segregation group			Total asci
		6 plus A 2 minus B	4 plus A ^a 4 minus B	8 minus B	
17	8-A-6 \times 8-A-3	1	6	0	7
37	30-A-4 \times 17-F-5	0	16	19	35
Total		1	22	19	42

^a Based on limited tests, plus cultures from asci with the 4:4 segregation were identical with the parent plus A strain as ascospores from them produced cultures of the plus type and of the minus A strain.

thecia in glomerate masses, and ascospores from these perithecial masses when isolated, produced cultures of the plus type and of the minus A strain. The subcultures of the latter were identical with the original minus A strain. Cultures of the minus B strain were not obtained from these subcultures.

On account of the occurrence of the minus B ascospores in asci with the 4:4 segregation along with ascospores of the plus type which produced cultures that segregated into the plus A and the minus A, there seems little doubt but that a fertilization did occur between the plus A and minus B strains.

Plus B and minus A.—Two cultures, the original plus B and an ascospore subculture which seemed identical, were mated with cultures of the minus A strain. Ridges of perithecia formed rapidly on the lines of contact.

The ascospores from 35 asci from these ridges were picked up and cultured (table 3). Of these asci, 17 had 4 spores of the plus type and 4 of the minus A strain, 11 had spores of the minus A strain alone, and 7 had spores of the plus type alone.

From 11 cultures of the plus type obtained from asci with the 4:4 segregation, 435 ascospores were cultured. The cultures from these were all of the plus type.

Plus B and minus B.—The original plus B culture was grown in plates with two cultures of the minus B strain. Ridges of perithecia formed quickly on lines of contact.

The ascospores from 26 asci from perithecial ridges were picked up and cultured (table 4). Of these asci, 8 had 4 spores of the plus type and 4 of

the minus B strain and 18 had spores of the minus B strain alone.

From 13 cultures of the plus type obtained from asci with the 4:4 segregation, 505 ascospores were cultured. The cultures from these were entirely of the plus type. From this it would seem that the spores of the plus type in the asci with the 4:4 segregation were of the plus B strain.

Discussion.—By using two strains of the plus type which differed in possibly only a single character and two unlike strains of the minus type, it was possible to demonstrate that fertilization between plus and minus strains does take place when they come in contact. From perithecia in the ridge of perithecia on the line of contact, it was always possible to obtain asci which contained four ascospores of each strain used in the cross. The cultures obtained from these ascospores were identical with one or the other of the parent strains. No new strains were observed in the crosses in these tests. This might be expected if the strains differ in only a single character. However, the facts available regarding the nuclear activities are so limited that it is not possible to offer any explanation at the present time.

The variant (plus B) of the plus type was of considerable interest genetically as none of the ascospores when isolated produced cultures of the minus type. This variant behaved like a homothallic fungus and if its origin from the ordinary plus type were not known, its behavior would probably be considered normal and in line with many other ascomycetes which have previously been studied by others. But originating from the progeny of a culture which produces ascospores of both the plus and minus types,

TABLE 3. *Asci in segregation groups from crosses between plus B and minus A strains.*

Cross number	Parents Plus B \times minus A	Number of asci in each segregation group			Total asci
		8 plus	4 plus B ^b 4 minus A	8 minus A	
23	5-16 \times 15-24-D-8	6	3	8	17
31, 34, 38	5-16-170 \times 4-17-1	1	14	3	18
Total		7	17	11	35

^b Plus cultures from asci with 4:4 segregation were identical with the parent plus B strain, as ascospores from them produced cultures of the plus type only.

TABLE 4. *Asci in segregation groups from crosses between plus B and minus B strains.*

Cross number	Parents Plus B \times minus B	Number of asci in each segregation group		Total asci
		4 plus B ^c 4 minus B	8 minus B	
12	5-16 \times 8-A-3	4	10	14
33	5-16 \times 17-F-5	4	8	12
Total		8	18	26

^c Plus cultures from asci with the 4:4 segregation were identical with the parent plus B strain, as ascospores from them produced cultures of the plus type only.

its nuclear constitution is not clear and at this time no attempt is being made to explain it. However, for that matter, there is no satisfactory explanation of the condition found in the ordinary plus strain where a culture originating from a single ascospore seems to segregate into two strains.

SUMMARY

From a culture of *Glomerella* from *Ipomoea* there were obtained two unlike strains of the plus type and two of the minus type.

A ridge of perithecia formed rapidly on the line of contact when either plus strain was grown with either minus strain.

From each cross, asci were obtained with four ascospores of the plus and four of the minus types used and others which contained eight ascospores of

the minus type used. This indicates that a fertilization between the two strains occurs.

A plus strain ordinarily seems to segregate into plus and minus. However, a strain of the plus type (plus B) was found which did not segregate in this way.

DEPARTMENT OF BOTANY,
LOUISIANA STATE UNIVERSITY,
BATON ROUGE, LOUISIANA

LITERATURE CITED

- EDGERTON, C. W. 1914. Plus and minus strains in the genus *Glomerella*. Amer. Jour. Bot. 1:244-254.
LUCAS, G. B., S. J. P. CHILTON, AND C. W. EDGERTON. 1944. Genetics of *Glomerella*. I. Studies on the behavior of certain strains. Amer. Jour. Bot. 31:233-239.

GROWTH AND VASCULAR DEVELOPMENT IN THE SHOOT APEX OF SEQUOIA SEMPERVIRENS (LAMB.) ENDL. I. STRUCTURE AND GROWTH OF THE SHOOT APEX¹

Clarence Sterling

IN RECENT years there has been a revival of interest in the classical problems of developmental anatomy. This renewed interest has resulted in part from new methods in histological technique, in part from the criticism of older concepts of shoot structure. Generally, however, the recent histogenetic studies have not been coordinated ones. They have been concerned with the development of a particular tissue or organ of the plant. What seems desirable is a coordinated treatment of the modes of growth in the organs and tissues which have been the objects of "classical" consideration.

It is with this idea as a basis that the present study of shoot organization in *Sequoia sempervirens* has been initiated. Although investigations have been made on the apical meristem of this species, its phyllotaxis, initial vascular differentiation, secondary cambial activity, and leaf anatomy, these studies generally are not unified. They present only

a limited picture of the interrelated activities in the growing redwood shoot. The present study has attempted to present a unified treatment of development, based on the use of modern histological techniques. Physiological speculations, with the awareness of the inherent limitations, are derived from the appearance of fixed and stained material.

The first paper of this series considers the development of the shoot apex above the region of vascular differentiation, development of the pith region of the stem, and general shoot morphology.

MATERIALS AND TECHNIQUES.—Several hundred shoot apices of leading sprouts of redwood were collected in Sequoia Park, Oakland, California. Apices of lateral branches were also gathered for comparative study. The plants from which these apices were taken were approximately five years old and from 1 to 2.5 meters high. For the year studied, the average height increment was 33 centimeters, some individual shoots attaining a growth of 63 centimeters. All were obtained within an acre of redwood grove, and many were probably geneti-

¹ Received for publication October 26, 1944.

The writer is indebted to Professor A. S. Foster for inspiration and critical advice in the development of this study.

cally related, in view of the sprouting habit of older *Sequoia sempervirens* trees.

The collections were begun at the end of February, 1941, and continued through January, 1942. Specimens were taken at weekly intervals during the season of active growth, from February through August, and at bi-weekly and monthly intervals during the limited dormant period, starting about September.

The apices were killed and fixed principally in a solution of the Craf type. A modification of Juel's solution was used occasionally to give better fixation of the walls of sieve cells. Following the method of Ball (1941a), the apices were dehydrated and ultimately embedded in paraffin. Sections were cut serially from 8μ to 10μ thick, in transverse, median longitudinal, and tangential planes. The most successful staining technique employed was the tannic acid-ferric chloride and safranin schedule of Foster (1934), modified by the use of a fast green counterstain.

STRUCTURE AND GROWTH OF THE SHOOT APEX.—On the leading shoots the leaves are small, linear, and arranged in phyllotaxes of varying complexity. They are decurrent, and ridges extend vertically along the stem from the sides of the leaf insertion until they reach the next leaves below. At points where an arrest in growth has occurred, the leaves, which here may be called cataphylls, are shorter and are crowded closely together, marking the termination of active growth.

Cytology of the active shoot apex.—Regarding the internal organization of the apical region of *Sequoia*, Cross (1943a) and Crafts (1943), respectively, have discussed apical histology and vascular organization. The present study, conducted independently of the above investigations, brings forth some additional, and on some points, divergent observations. It is hoped that these findings may contribute to a better evaluation of *Sequoia* with respect to conifer anatomy and general shoot structure. An extensive critique of works on apical structure seems needless because of the excellent reviews of Foster (1939b, 1941a).

The following description, based on the study of median longisections and transections, briefly represents the cytological situation in the shoot apex.

The summit of the apical cone in an active shoot is occupied by a group of three or four cells, which are slightly smaller, more deeply-staining, seemingly less vacuolate,² and more actively dividing than their derivatives. This group of cells is perpetually meristematic, constituting an irregular zone which may be designated as the "apical initials" (fig. 6, 7). This zone is thus the ultimate generative meristem of the apex.

The apical initials divide periclinally and anti-

² Vacuolate is used in reference only to the combined effect of the lightly-staining quality of the cytoplasm with the relatively larger size of the vacuoles. It is recognized that vacuoles occur in all cells, including those of the meristem (Bailey, 1930; Zirkle, 1932).

clinally, irrespective of season of growth, and oblique cross walls also occur. Almost every shoot apex examined showed at least one periclinal wall in one of the cells of this zone. There is no regularity in the sequence of anticlinal and periclinal

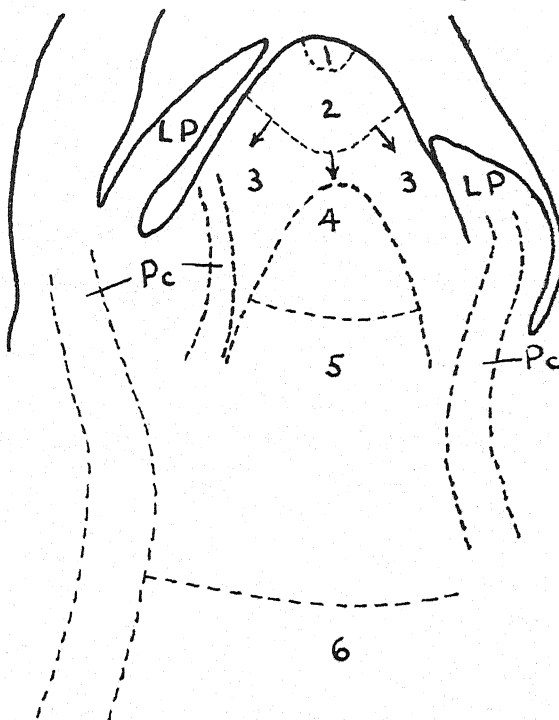


Fig. 1. Diagram, based on figure 3, of a longisection of the shoot apex of *Sequoia sempervirens*, showing the pattern of cytohistological zonation and tissue differentiation. Zone 1 is constituted by the apical initials. The derivative zone immediately below (zone 2) is the area of central mother cells. From the flanks and base of zone 2, a eumeristem zone (zone 3) is produced by renewed mitotic activity. The pith mother cells (zone 4) are derived from eumeristem cells in the center of the shoot. From the eumeristem cells on the flank are differentiated epidermis, procambial strands (Pc), leaf primordia (LP), and cortical tissue. In the pith, the pith mother cells eventually give rise to a rib-meristem (zone 5), beneath which the cells of the pith experience elongation in the transverse plane—zone of transverse expansion (zone 6).

divisions. Although anticlinal divisions tend to predominate in the anticlinal derivatives of the apical initials, thus producing an apparently discrete surface layer, periclinal and oblique walls are abundant enough in these derivatives on the flanks of the cone to invalidate the idea that a discrete "protoderm" exists in this species (fig. 3, 6, 10).

Strasburger (1872) declared that the *Sequoia* apex (in addition to *Taxus*, *Ginkgo*, *Thuja*, *Cryptomeria*, and others) had an independent protoderm overlying a layer or two of periblem cells. He admitted, however, that there were occasional divisions in the protoderm, as in *Ephedra*. Campbell (1940) figured the *Sequoia sempervirens* apex without describing it. His legend seems to indicate

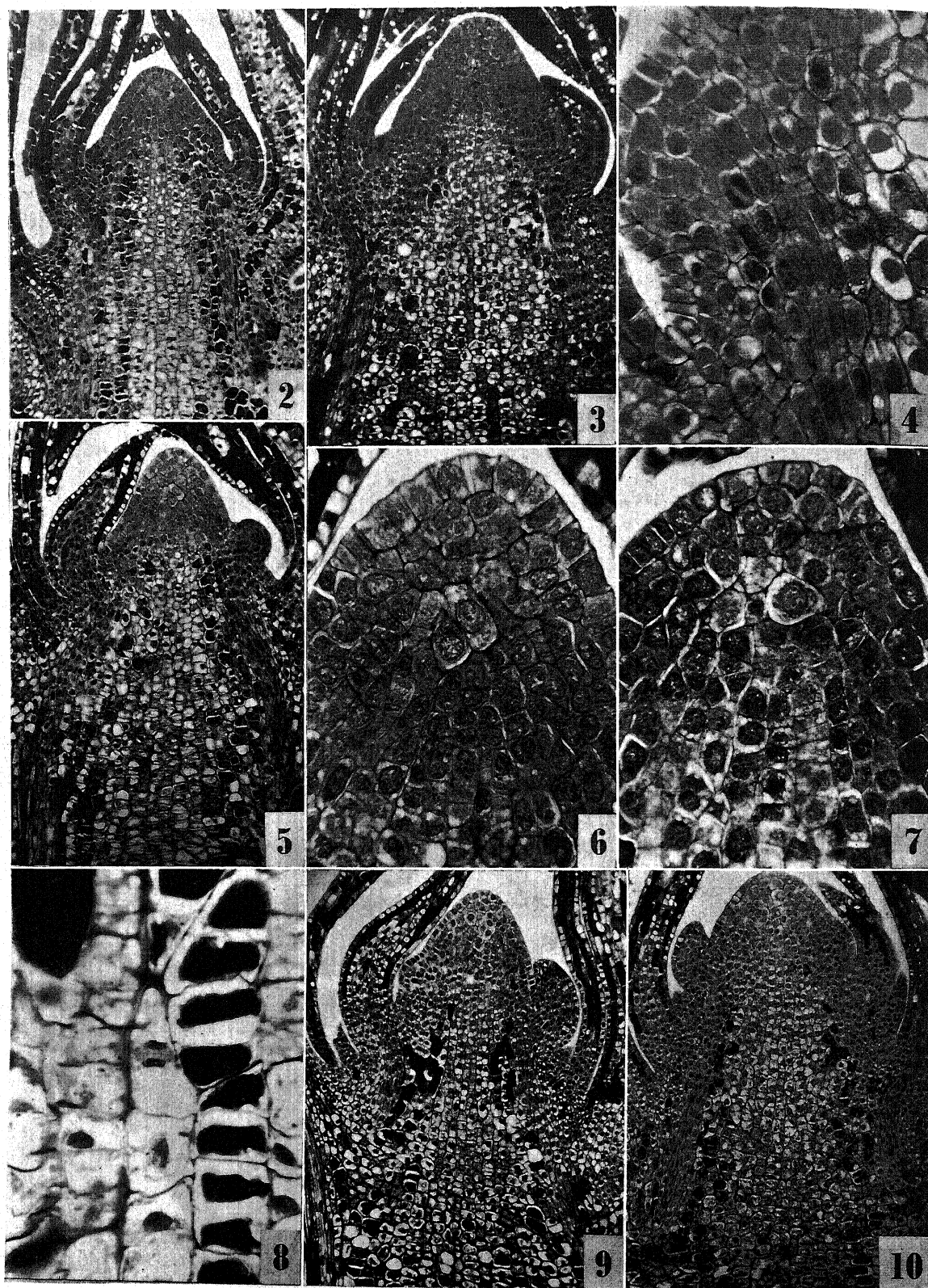


Fig. 2-10.—Fig. 2. Median longisection of active shoot apex of *Sequoia sempervirens* during period of most rapid shoot elongation. Note abundant mitoses in the eumeristem and pronounced, although delayed, development of an active

that he is in substantial agreement with Strasburger on the construction of that apex. Cross (1943a) finds that a protoderm exists seasonally in the shoot apex of *Sequoia sempervirens*, principally during the period of dormancy. These observations contrast significantly with the present finding, which has had the combined advantages of modern histological techniques and a seasonal collection of even-aged shoots from a small geographic area.

The derivatives of the apical initials form a cup-shaped region of lightly-staining cells. These cells are significantly larger than their own immediate derivatives. The walls are thicker also, and there seem to be large deposits of some substance at the wall interstices. Moreover, the frequency of mitoses is much lower than in the lateral and immediately subjacent cells. Collectively, these characteristics define a rather distinct zone in the shoot apex which may be called a "central mother cell zone." In this zone, divisions occur in all planes. Primary pit fields are readily visible in the walls of the central mother cells. In many instances the thickened wall of a mother cell can be seen to enclose several thinner-walled daughter cells. The nuclei of these cells are larger, stain less densely, and occupy a smaller proportion of the cell volume than the nuclei of the more active cells of the next lower region (fig. 6, 7).

The cells of the subjacent zone, called "peripheral meristem" by Cross (1943a) and cytologically equivalent to Kaplan's (1937) "eumeristem,"³ are smaller and more deeply staining in cytoplasm and nuclei, have thinner walls (with no interstitial deposits), and undergo a far higher frequency of division than the central mother cells. The difference between the sizes of the cells in the two zones can be as much as 2:1 and, not infrequently, 3:1. This eumeristem occurs directly below and lateral to the central mother cell zone (fig. 6, 7). It is derived abruptly from the latter by a renewal of mitotic activity. The cells of the eumeristem divide mainly by walls parallel and perpendicular to the boundary of the central mother cell zone, as seen in median longisection and in transection. At the center of the shoot apex, the eumeristem differen-

tiates into the pith mother cell zone, which farther down gives rise to the enlarging and dividing rib-meristem. Between the central mother cells and the pith mother cells, the eumeristem can vary from one to five cells in depth, depending on shoot activity.

Enveloping the pith mother cells and making up the remainder of the body of the shoot at this point is eumeristem which has not yet undergone differentiation. It is equivalent to Sanio's (1863) "Aus-senschicht," Korody's (1937) "Meristemglocke," and Foster's (1938, 1940) "peripheral zone"; it has been noted in many shoots in which the pith differentiates early. From this tissue are derived leaf primordia, cortex, vascular tissue, and epidermis.

The form of the apex above the level of insertion of the youngest leaf, as seen in median longitudinal view, is generally parabolic. This parabola may vary greatly in width, resulting in hemispherical to acutely triangular apices. These variations in form do not appear to be seasonal. Regardless of shape, leading shoot apices generally show the same topographic location of the cytohistological zones. Differences occur mainly in the relative proportions of the apex occupied by the various zones. For example, the hemispherical form shows a predominance in anticlinal divisions along the periphery of the apex (tending to form a surface layer over it) and a decrease in the axial⁴ extent of the central mother cell zone. The triangular apex shows a great many periclinal divisions in the apical initial group and an increased development axially of the central mother cell zone.

It is interesting to note, in this connection, that the majority of lateral branchlet apices studied were characterized by the hemispherical apex and the accompanying features noted above. Also, in these apices maturation of the pith cells takes place earlier than in the leaders (observed also by Crafts, 1943), and there is not so extensive a development of rib-meristem—see discussion on pith, below.

Origin and growth of the pith.—The pith mother cells are larger and more lightly-staining than their predecessors in the eumeristem. They divide frequently but not as often as the surrounding cells

³ The term "eumeristem" is employed in this paper in a cytological sense. Eumeristem tissue is composed of eumeristem cells.

⁴ The term "axial" is used throughout to indicate a direction parallel to the vertical axis of the shoot.

rib-meristem in the pith. $\times 108$.—Fig. 3. Median longisection of active shoot apex later in the growing season than apex of figure 2. Observe the decrease in frequency of cell divisions and the start of wall thickening in the zone of transverse expansion in the pith. $\times 108$.—Fig. 4. Median longisection of emergent foliar primordium with its procambial strand. Note development of light-staining leaf gap area in future axillary region. $\times 390$.—Fig. 5. Median longisection of shoot apex near end of period of active growth, showing well-defined central mother cell zone. The bud primordium on the right flank of the apical cone is supplied with a continuous procambial strand. A lightly-staining "gap" area can be seen immediately above this primordium. $\times 108$.—Fig. 6. Summit of apex of figure 5 at higher magnification. Note sharp boundary between central mother cell zone and eumeristem region, which is due to difference in size of cells in these two zones as well as difference in their staining properties. A periclinal division is observable in a surface cell at the left flank of the apical cone. $\times 390$.—Fig. 7. Median longisection of summit of apical cone of an active shoot. The ginkgoid nature of the cytohistological zones is readily observable. $\times 390$.—Fig. 8. Longisection through zone of axial elongation in the pith, showing mitoses in two superposed cells about 2500 microns below the summit of the apical cone. $\times 390$.—Fig. 9 and 10. Median longisections of dormant shoot apices. The zone of transverse expansion is well developed. Rib-meristem differentiates almost directly from the central mother cell zone. $\times 108$.

of the eumeristem "mantle." Divisions take place in all planes, but the tendency exists for a predominantly axial orientation of the spindle figure. This tendency becomes stabilized in the developing rib-meristem—usually located at some distance below in the active shoot. It is to be noted, in this connection, that there is no sharp demarcation between the pith mother cells and the rib-meristem. The level at which transverse divisions become prominent in the pith region varies with the seasonal activity of the shoot. In a vigorous shoot, the typical rib-meristem develops relatively far down in the pith and has a very extensive depth of activity. In the less active and dormant shoots, the rib-meristem is virtually coincident in appearance with the zone of pith mother cells. Hence, in the pith of these latter shoots, there are no randomly-oriented divisions such as characterize the pith mother cells.

In the pith mother cells and their derivatives, the primary wall begins to undergo some thickening. Also it is not unusual for isolated cells to accumulate a heavy deposit of some ergastic substance, possibly tanniferous in nature.

The individual cells experience a definite sequence of size increase immediately below the pith mother cells. First, there is an equilateral or isodiametric expansion of the daughter cell until it is approximately twice or three times the original mother cell's linear dimensions. The cell is still isodiametric or approximately so. About 1 mm. below the tip of the shoot, a transverse expansion sets in gradually. There is no decrease in cell height. The amount of expansion generally is to about two or three times the former cell width (fig. 1, 3, 5, 9, 10). This "zone of transverse expansion" is of variable extent (depending on the vigor of the shoot growth), ranging from $\frac{1}{2}$ to 2 mm. in depth. Generally this expansion establishes the ultimate width of the pith in the stem. At this level, the growing region of the stem has been widened by the resultant tissue expansion, and even the procambial strands can be seen to be bowed outwardly. The expansion is limited to the cells of the pith—it is absent from the cortex.

A vertical elongation then takes place which re-establishes the original isodiametric form of the cell (fig. 8). This elongation is achieved at about 4 mm. below the summit of the shoot. The combined effect of transverse expansion and vertical elongation is to change the original polyhedral form of the pith mother cells to a much larger cylindrical one, in which the height is equal approximately to the diameter of the base.

The pith in the leading shoot of *Sequoia* shows some interesting features which render it rather unique among conifers. As Cross (1943a) has mentioned, there is a very much delayed maturation of the pith cells. This seems to be true also of *Cunninghamia*, as observed by Cross (1942). Marked vacuolation, as a rule, occurs only below the pith mother cells, and nowhere does it ever reach above the level of insertion of the youngest leaf primor-

dium. The cells gradually become vacuolate in the pith proper, with few immediately attaining full size or becoming filled with ergastic material. In the active shoot, the rib-meristem region extends far down in the pith.

Mitoses occur throughout the rib-meristem zone and are present in the zone of transverse expansion. They are rarer in the zone of vertical elongation but do occur at relatively great distances below the summit of the apical cone (2 and 3 mm. in many apices). Many dividing cells in this zone of vertical elongation show the development of the kinoplasmosome (its fibrils are clearly delineated at the edge of the growing cell plate) in the formation of the new cell plate. (See Sinnott and Bloch, 1941). Virtually all these mitoses have the long axis of the spindle figure oriented parallel to the shoot axis (fig. 8).

Origin and development of foliage leaves.—At the shoot apex, the leaves arise in a sort of "rosette," as Koch (1891) calls it, about the lower flanks of the apical cone. The apex is devoid of protuberances for about $130\ \mu$ to $180\ \mu$ below the tip. The primordia arise in predetermined order in various phyllotactic series. (The word "predetermined" is used advisedly, as will be seen in a later paper.) In terms of cellular pattern, the origin of a leaf primordium in *Sequoia* follows the same general procedure outlined by Cross in his studies of other taxodes. The surface cells at the point of emergence usually do not undergo periclinal divisions. The raising of the primordium from the buttress is accomplished by periclinal sub-epidermal activity (fig. 2, 4, 9). In a few instances, however, periclinal divisions have been observed in the surface layer of the emergent primordium. (This has been seen in occasional young bud primordia also.) These periclinal divisions in the surface layer are by no means prominent, and some may actually be configurations evoked by a fortuitous oblique division cut perpendicularly by the microtome blade.

It is important to note that soon after the emergence of a primordium, the area between its axil and the pith mother cell region becomes vacuolate, seemingly in an acropetal direction. This vacuolated area appears to be the forerunner of the future leaf gap. Majumdar (1942) also observes the basifugal development of the gap area, and Esau (1942) shows early differentiation of this region in her figures 1, 2, and 4. This adaxial vacuolation is seen when the primordium is but $30\ \mu$ to $40\ \mu$ high and occurs in the eumeristem zone of the shoot apex itself (fig. 4, 5).

Possibly the procambial strand (which is present at an early stage) exerts some sort of physiochemical influence centripetally, causing the eumeristem cells proximal to this strand to differentiate (i.e., become vacuolate) when the primordium is but three or four cells high. This strip of vacuolate cells is possibly the earliest appearance of the foliar gap, which widens soon to provide a perma-

nent change in the course of differentiation of the vascular tissue.

The apex of the emergent leaf does not have the lightly-staining central mother cell zone so conspicuous in the shoot apex. The cells of the foliar apex all seem to consist of the eumeristem tissue from which they took their origin (fig. 2, 9, 10). Although initially growth seems to be impelled by hypodermal divisions (with the surface layer merely experiencing anticlinal divisions in this emergence), eventually apical growth of the primordium becomes localized for a very short time in a few surface cells. These cells undergo periclinal divisions principally, to give the leaf apex a pointed aspect which is maintained permanently by virtue of an early maturation of that apex. Subsequent to that maturation, the sub-apical cells at the tip and the cells in the middle of the leaf undergo active divisions to produce extensive intercalary growth. (cf. Cross, 1940, 1942).

Origin and development of bud primordia.—Because of the crowded appearance of the primordia in longisection, only two criteria have been found for the determination of the nature of the emergent primordium. One criterion is the direction of growth, and the other is the form of the new member. The nascent leaf primordium develops a large buttress and, when only a few cells high, begins to grow upwardly. The emergent bud primordium, however, develops laterally. In addition, the bud assumes a hemispherical form when about five cells high (fig. 5). At the same height, the leaf primordium is already starting to develop a pointed apex (fig. 10, on left flank of apical cone).

In transection, the young leaf primordium is circular while the emergent bud primordium is spindle-shaped, its long axis parallel to the tangent of the shoot at the point of emergence. The first appearance of the bud is an undifferentiated mass of highly meristematic cells in the axil of the subtending leaf. At its earliest recognizable stage, it is already lenticular in cross-section. As the bud meristem enlarges, two leaf primordia differentiate at opposite ends of the long axis of the "spindle" to become the prophylls of the new bud.

In contrast to the unstratified nature of the shoot apex, leaf and bud primordia initially grow with a discrete surface layer. As noted earlier, however, occasional periclinal divisions may occur in both structures.

Longisections occasionally show tissue zones of vertically elongated cells. These cells are seen in the form of arcs curving about the base of the bud primordium, their concave "surface" facing the primordium. Configurations such as this have also been noted in the apical cone without reference to the presence of bud primordia. The cells of this tissue are tabular, like those of the procambium, but appear to be unconnected with the procambium of the leaf traces in most cases.

In transection there can be seen zones of cells in the axils of both leaf and bud primordia which may be related to the tissue zones described above.

These cells are tangentially elongated and are arranged in the form of an inwardly bowed arc. The cells stain densely and soon acquire thick walls. The tissue extends inward and downward into the shoot proper, disappearing a few hundred micra below the axillary region.

Schmidt (1924) has called attention to the "shell-like" zones associated with the emergence of bud primordia. These zones are said to be non-procambial tissue zones of elongated cells (as seen in longisection) which curve about the base of the primordium, serving to raise the primordium by successive tangential divisions. They are described as being "akrofulgal" in development. Koch (1891) and Esau (1942) have mentioned similar cell configurations seen in the shoot apex, although not in respect to bud primordia, and have emphasized their transitory nature. Reeve (1943) has described the shell-like zone in *Garrya* in connection with the raising of the axillary bud and has shown the zone's independence of the procambium. (See also Hsü, 1944, fig. 11, 14).

Although the cell pattern described above seems to be related to axillary bud emergence in the dicotyledons studied by Reeve (1943) and Schmidt (1924), there does not seem to be such a connection in the studies of Koch (1891) on conifers, of Esau (1942) on *Linum*, or of the present investigation on *Sequoia*. Further investigation remains to be undertaken to clarify the relationship of this cell pattern with the other growth processes in the shoot apex.

Cytology of the "dormant" shoot.—The above observations have referred to the active shoot apex. During the active growing season, approximately from March through September, leaves are produced in rapid succession. The approach of dormancy brings few changes to the structure of the terminal bud. One significant change is the transition from foliage to cataphyllary leaves about June or July. The cataphyll is marked by an early cessation of intercalary growth. Also, the epidermal cells seem to be vacuolate at an early stage. Vascular tissue is present, as is also transfusion tissue. The mesophyll parenchyma matures early, and its cells become filled with ergastic material. On the whole, the cataphylls appear to resemble foliage leaves arrested during their development (fig. 9 and 10).

The fact that dormancy in *Sequoia* is incomplete is also seen in the occurrence of mitoses throughout the apex and even in the cataphylls of dormant buds collected as late as December and January. Although it is true that these mitoses are rare and that the majority of cells have spherical, resting nuclei, still the presence of mitoses does indicate a growth activity in *Sequoia* that persists throughout all seasons. The fact that the climate in the San Francisco Bay area is comparatively mild the year round may account for the pseudo-dormancy of the apex.

Another conspicuous feature of the dormant shoot is the heavy thickening of the pith cell walls in the

region of transverse expansion (fig. 9, 10). This thickening, anisotropic in polarized light, occurs coincidentally with the formation of cataphylls by the shoot apex. At the conclusion of the wall-thickening process, the walls of these cells are about ten times their previous width and resemble the walls in the "Kollenchymplatte" (Korody, 1937) or "crown" of dormant abietinous buds. (See also Busse, 1893; Lewis and Dowding, 1924; Kemp, 1943, for comparable observations and speculation upon the nature of wall thickening occurring in the zone of transversely expanded cells.)

Slightly before the walls begin to thicken, the cells of this zone expand rather abruptly in the transverse plane, and to a greater extent than in the active shoot, so that the width of the cells becomes three or four times greater than the height. The vascular strands about this zone become conspicuously bowed. The pith cells located above this zone maintain their normal aspect and do not undergo any notable wall thickening. The wall thickening is initiated in the zone of transverse expansion and extends downward for about 1 mm. below that zone. Farther down, the pith cells are thin-walled. Ergastic substances are abundant in the cells of the pith throughout all its extent, including the zone of transverse expansion. They are present to a lesser extent in the pith mother cells and the very young rib-meristem.

With increasing dormancy, the rib-meristem tends to become more limited in extent and differentiated higher and higher in the shoot until, in the fully dormant apex, this tissue is virtually coincident with the eumeristem (fig. 9, 10). It will be recalled that at the beginning of spring growth, however, the rib-meristem is differentiated only far below the pith mother cell zone and that the zone of transverse expansion is but partially developed (fig. 2, 3, 5-7). The observations on these pith zones leads to the necessary conclusion that there is a relative, rather than absolute, point of inception and extent of development of the various zones in the apex. Thus, the more vigorous the shoot growth, the less is the amount of transverse expansion and the more the amount of vertical elongation, accompanied by a greater, although delayed, development of the rib-meristem. Conversely, the onset of dormancy produces the opposite tendency.

Finally, it is important to note that in the dormant bud of *Sequoia*, there is no telescoping of the shoot of the following season, as occurs in *Torreya* and the *Abietaceae*. The apex of the dormant *Sequoia* bud presents the appearance of having been arrested during the process of active growth (fig. 9, 10). As in *Pinus montana* (Korody, 1937), the apex appears the same the year round; there are no differing stages in the general morphology of the shoot apex during the growing season.

DISCUSSION.—Cross (1939, 1941, 1942, 1943a, b) has discussed the structure of the shoot apex in the *Taxodiaceae*, reviewing the older works on the subject at the same time. He comes to the conclu-

sion that the apex consists, in general, of an apical initial group giving rise to a protoderm by anticlinal divisions. From the sub-apical mother cells there are produced a cylinder of peripheral meristem and a core of pith mother cells. Cross (1943a) stresses that very occasional periclinal divisions may occur in the *Sequoia* "protoderm" on the apical flanks and that in this species the frequency of periclinal divisions in the apical initials appears to fluctuate seasonally. Cross (1943b) also indicates that in all the *taxodes* the pith mother cells (in the form of small files of rib-meristem) differentiate directly from the sub-apical mother cells.

It is evident from the present findings that there is no indication of an independent protoderm (except in lateral buds) in the shoot apex of *Sequoia sempervirens*. Periclinal divisions have been found in the apical initials and along the flanks of the apical cone in great enough numbers to invalidate this interpretation. The anticlinal derivatives of the apical initials may be said to have a tendency toward forming a discrete layer, but this tendency is not often fully expressed.

In connection with the relationship between sub-apical initials and pith mother cells, Cross' description of the leading shoot apex of *Sequoia* seems to correspond to the writer's observations on the apices of lateral branches. Cross' illustrations show vacuolated pith immediately below the sub-apical mother cells. The present work gives an entirely different picture of tissue organization in the apex of leading shoots: there is a lightly-staining zone of rather inactive, large cells with thick walls and heavy deposits in the cell interstices occupying the tip of the shoot beneath the apical initials. Below and laterally, this zone produces small cells, with denser cytoplasm, deeply-staining nuclei, higher nucleocytoplasmic ratio, and thinner walls—the typical eumeristem of Kaplan (1937). The whole aspect of central mother cells and eumeristem is suggestive of the situation in *Ginkgo* (Foster, 1938), the cycads (Foster, 1939a, 1940, 1941b, 1943), some cacti (Boke, 1941), *Phoenix* (Ball, 1941b), and *Sinocalamus* (Hsü, 1944).

Foster (1938, 1941a) has analyzed the ginkgoid situation as differing from that of the "typical" conifer in that the undifferentiated sub-apical mother cells in the latter develop into pith mother cells by progressive vacuolation, whereas in *Ginkgo*, the central mother cell group represents an interpolation between the apical initials and the active rib-meristem of the pith. The same, in more exaggerated development, was found true in the cycads (Foster, 1939a, 1940, 1941b, 1943). Taken from this point of view, the *Sequoia* apex preserves more of the ginkgoid structure than do other conifers described in recent cytological treatments. Its structure might be considered intermediate between the ginkgoid and abietinous types.

By contrast, Cross (1943a, b) found in *Sequoia* and other *Taxodiaceae* that the sub-apical mother cells could be distinguished from their neighbors

only by position, isodiametric shape, and irregular planes of division—in *Sequoia* the walls of the mother cells were often irregularly thickened. Although the *Sequoia* apices in the present investigation differ in the extent to which the different zones are developed, in the majority of them the central mother cell zone has the distinctive features noted in *Ginkgo*.

The delayed differentiation of the pith, i.e., delayed vacuolation and retarded development of the rib-meristem, in active shoot apices of *Sequoia* is apparently uncommon among other conifers. The most nearly related condition reported in the literature is that of *Cunninghamia* as explored by Cross (1942). (This species also resembles *Sequoia* in the abundance of periclinal cells in the apical initials and the comparative inactivity of the sub-apical mother cells.) Contrary to Cross' (1943b) conclusion that the Taxodiaceae differ from the Abietaceae in possessing only a relatively short zone of rib-meristem, in *Sequoia sempervirens* this zone is very extensive and strongly developed and contributes, very likely, to the significant amount of growth in height that takes place during the year.

Various descriptions of the cell configuration in the shoot apex of *Sequoia sempervirens* occur in the literature. These descriptions range from that of an apical cell initial (Douliot, 1890) to that of an independent continuous protoderm layer overlying two layers of periblem (Campbell, 1940). Overemphasis on the mechanics of cellular configuration at the shoot apex and strict adherence to a theory of limited descriptive convenience have led some writers to homologize the whole conifer shoot apex with the inner portion of an angiospermous apex (Buder, 1928; Korody, 1937). This tendency has been criticized by Cross (1939) and Foster (1939b).

The fact that in conifers all variants of apical cell group aspects, from an apparently dominant apical cell to a seemingly discrete surface layer, may occur as transitory phenomena, would seem to deny the importance placed hitherto upon the specific cell arrangement in the shoot apex by various investigators. "Obviously," as Foster (1939b) says, "a more realistic cytological picture of the meristem is indispensable for any true insight into problems of growth and differentiation." However, even the application of the cytohistological approach to these problems must be undertaken with a clear idea of the variability within the plants studied. All the facets of behavior must be sought out by means of a comprehensive sampling of the material throughout the season of growth. Careful technical treatment is essential in preserving features which could be obliterated by crude processing.

SUMMARY

The apex of the leading shoot of *Sequoia sempervirens* has a ginkgoid zonation. Apical initials divide anticleinally and pericleinally to produce a central mother cell zone, the cells of which are

larger and more vacuolate, have thicker walls, and undergo fewer mitoses than the cells of the subjacent zone. This subjacent zone is composed of eumeristem cells which have arisen from the base and sides of the central mother cell zone by a renewal of mitotic activity. Pith mother cells differentiate from the eumeristem cells in the center of the shoot. The level of appearance and extent of development of the rib-meristem in the pith appears to depend upon the state of activity of the apex.

In dormant shoots there is a prominent "crown" of transversely expanded cells with thickened walls in the pith. This crown separates the apex from the rest of the shoot. There is no telescoping of the shoot of the following season in the dormant bud.

When the leaf primordium is but a few cells high, the future "gap" area begins to develop adaxially to the procambial strand of the primordium. Although the foliar and bud primordia both are produced principally by subepidermal pericleinal activity, the young bud maintains a hemispherical form in longisection in its early development while the nascent leaf develops a pointed apex when about five or six cells high. In transection, the bud primordium is shaped like a spindle, at each pole of which a prophyll arises.

DEPARTMENT OF BOTANY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

- BAILEY, I. W. 1930. The cambium and its derivative tissues. V. A reconnaissance of the vacuome in living cells. *Zeitschr. Zellf. u. Mik. Anat.* 10: 651-682.
- BAILL, E. 1941a. Microtechnique for the shoot apex. *Amer. Jour. Bot.* 28: 233-243.
- . 1941b. The development of the shoot apex and of the primary thickening meristem in *Phoenix canariensis* Chaub., with comparisons to *Washingtonia filifera* Wats. and *Trachycarpus excelsa* Wendl. *Amer. Jour. Bot.* 28: 820-832.
- BOKE, N. H. 1941. Zonation in the shoot apices of *Trichocereus spachianus* and *Opuntia cylindrica*. *Amer. Jour. Bot.* 28: 656-664.
- BUDER, J. 1928. Der Bau phanerogamen Sprossvegetationspunktes und seine Bedeutung für die Chimären Theorie. *Ber. Deutsch. Bot. Ges.* 46: (20)-(21).
- BUSSE, W. 1893. Beiträge zur Kenntniss der Morphologie und Jahresperiode der Weissstanne (*Abies alba* Mill.). *Flora* 77: 113-175.
- CAMPBELL, D. H. 1940. The evolution of the land plants (Embryophyta). Stanford Univ. Press. Stanford Univ., Calif.
- CRAFTS, A. S. 1943. Vascular differentiation in the shoot apex of *Sequoia sempervirens*. *Amer. Jour. Bot.* 30: 110-121.
- CROSS, G. L. 1939. The structure and development of the apical meristem in the shoots of *Taxodium distichum*. *Bull. Torrey Bot. Club* 66: 431-452.
- . 1940. Development of the foliage leaves of *Taxodium distichum*. *Amer. Jour. Bot.* 27: 471-482.
- . 1941. Some histogenetic features of the shoot apex of *Cryptomeria japonica*. *Amer. Jour. Bot.* 28: 573-582.

- . 1942. Structure of the apical meristem and development of the foliage leaves of *Cunninghamia lanceolata*. Amer. Jour. Bot. 29:288-301.
- . 1943a. A comparison of the shoot apices of the Sequoias. Amer. Jour. Bot. 30:130-142.
- . 1943b. The shoot apices of *Athrotaxis* and *Taiwania*. Bull. Torrey Bot. Club 70:335-348.
- DOULIOT, H. 1890. Recherches sur la croissance terminale de la tige des Phanérogames. Ann. Sci. Nat. Bot. VII. 11:283-350.
- ESAU, K. 1942. Vascular differentiation in the vegetative shoot of *Linum*. I. The procambium. Amer. Jour. Bot. 29:738-747.
- FOSTER, A. S. 1934. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. Stain Tech. 9:91-92.
- . 1938. Structure and growth of the shoot apex in *Ginkgo biloba*. Bull. Torrey Bot. Club 65:531-556.
- . 1939a. Structure and growth of the shoot apex of *Cycas revoluta*. Amer. Jour. Bot. 26:372-385.
- . 1939b. Problems of structure, growth and evolution in the shoot apex of seed plants. Bot. Rev. 5:454-470.
- . 1940. Further studies on zonal structure and growth of the shoot apex of *Cycas revoluta* Thunb. Amer. Jour. Bot. 27:487-501.
- . 1941a. Comparative studies on the structure of the shoot apex in seed plants. Bull. Torrey Bot. Club 68:339-350.
- . 1941b. Zonal structure of the shoot apex of *Dioon edule* Lindl. Amer. Jour. Bot. 28:557-564.
- . 1943. Zonal structure and growth of the shoot apex in *Microcycas calocoma* (Miq.) A. DC. Amer. Jour. Bot. 30:56-73.
- Hsü, J. 1944. Structure and growth of the shoot apex of *Sinocalamus Beecheyana* McClure. Amer. Jour. Bot. 31:404-411.
- KAPLAN, R. 1937. Über die Bildung der Stele aus dem Urmeristem von Pteridophyten und Spermatophyten. Planta 27:224-268.
- KEMP, M. 1943. Morphological and ontogenetic studies on *Torreya californica* Torr. I. The vegetative apex of the megasporangiate tree. Amer. Jour. Bot. 30:504-517.
- KOCH, L. 1891. Ueber Bau und Wachstum der Sprossspitze der Phanerogamen. I. Die Gymnospermen. Jahrb. f. wiss. Bot. 22:491-680.
- KORODY, E. 1937. Studien am Spross-Vegetationspunkt von *Abies concolor*, *Picea excelsa* und *Pinus montana*. Beitr. z. Biol. d. Pflanzen 25:23-59.
- LEWIS, F. J., AND DOWDING, E. S. 1924. The anatomy of the buds of Coniferae. Ann. Bot. 38:217-228.
- MAJUMDAR, G. P. 1942. The organization of the shoot in *Heracleum* in the light of development. Ann. Bot. N.S. 6:49-82.
- REEVE, R. M. 1943. Comparative ontogeny of the inflorescence and the axillary vegetative shoot in *Garrya elliptica*. Amer. Jour. Bot. 30:608-619.
- SANTO, C. 1863. Vergleichende Untersuchungen über die Zusammensetzung des Holzkörpers. Bot. Zeit. 21:357-363, 369-375, 377-385, 389-399, 401-412.
- SCHMIDT, A. 1924. Histologische Studien an phanerogamen Vegetationspunkten. Bot. Arch. 8:345-404.
- SINNOTT, E. W., AND R. BLOCH. 1941. Division in vacuolate plant cells. Amer. Jour. Bot. 28:225-232.
- STRASBURGER, E. 1872. Die Coniferen und die Gnetaceen. Hermann Dabiz. Jena.
- ZIRKLE, C. 1932. Vacuoles in primary meristems. Zeitschr. Zellf. u. Mik. Anat. 16:26-47.

THE CALCULATION OF TENSIONS IN CUCURBITA PEPO¹

C. Ralph Stocking

IN SPITE of the occasional objections raised against it (Peirce, 1936; Priestley, 1935; Woodhouse, 1933), the cohesion theory of water movement in plants, based on the classical researches of Dixon and Joly (1894) and Askenasy (1895), has gained almost universal acceptance by plant physiologists. The theory as generally accepted proposes that water is able to move through plants by reason of its cohesion and continuity and that such movement is conditioned by the existence of factors causing differences in the diffusion pressure (Meyer, 1938) of water between points in the continuous water mass of the plant body and its immediate environment: the soil and the atmosphere. Forces of cohesion in the water and of adhesion between the water and the walls permit the maintenance of liquid continuity under conditions of greatly reduced pressure and prevent by the action of surface tension the penetration of undissolved

gas through wet cellulose walls. Often there is set up a state of reduced pressure or tension in the non-living xylem elements extending downward from the leaves. Crafts (1939) has pointed out that water under tension in the xylem is in a metastable state, the stability of the system depending upon the fact that there are no unwet surfaces upon which a vapor phase may be initiated.

Difficulties in technique have thus far prevented the direct measurement of tensions which according to this theory are developed within the xylem. The use of manometers attached directly to cut branches, stumps, and stems entails a disruption of the conducting tracts, and even when special precautions are taken to remove all gas from the tissue the maximum tensions recorded have been less than 2 atmospheres (Böhm, 1892; Thut, 1932). Dixon (1924) calculated that tensions of at least 23 atmospheres were required to raise water in tall trees while dendrographic experiments have been cited as indicating the probable existence of tensions as high as 200 atmospheres (MacDougal, Overton and Smith, 1929). Preston (1938) calculated from the rate of

¹ Received for publication November 11, 1944.

The author is indebted to Dr. A. S. Crafts for his interest and guidance, to Dr. F. Veilmeyer for helpful criticism, and to the Division of Botany, University of California at Davis, for research facilities during the course of this investigation.

injection of dye solutions into an ash stem that the tensions were of the order of 3 atmospheres.

Renner, using potometers (1911, 1912), estimated the normal tensions to be 1.0 to 1.5 atmospheres in well watered plants with an extreme of 5 atmospheres, 10 to 20 atmospheres in wilted leaves and 1 atmosphere in plants growing in culture solution. Nordhausen (1921), unable to place confidence in these results, used a similar but modified technique and reported tensions of 3 to 4 atmospheres in trees and 7 to 8 atmospheres in cut shoots. Recently Eaton (1941) used a modification of Renner's technique and reported an average tension of 3.55 atmospheres for a water culture plant.

Estimates of tensions based on a knowledge of the rates of exudation from stumps under known sub-atmospheric pressures and of the rates of transpiration of intact plants were made by Warne (1942). These estimates varied, ranging from tensions, under conditions of high transpiration, of 1.21 atmospheres for *Pelargonium* to 13.12 atmospheres for *Erica*.

Holle (1915) pointed out that vessels in wilted leaves of *Alliaria officinalis* were full of water as shown by direct observation and contended that under such conditions the tension of the vessel sap must be equal to the osmotic pressure of the mesophyll cells. Numerous plasmolytic measurements reporting values of 0 to 20 atmospheres have been made of "suction tensions" of cells in various plant parts (Beck, 1928; Ursprung, 1935) but have been severely criticized (Ernest, 1934; Oppenheimer, 1930).

This paper is the result of an investigation designed to develop a new method of calculating the magnitudes and fluctuations of tensions produced in the xylem of intact squash plants during normal growth in the field as well as in culture solutions and under conditions of extreme wilting.

MATERIALS AND METHODS.—The experiments described were conducted with Hubbard and Danish squash plants, *Cucurbita pepo*, grown in water culture and in soil. Modified Hoagland's solution containing traces of minor elements was used. The soil used for soil-grown plants was a light greenhouse mixture of Yolo loam, sand and peat. Some experiments were performed on field-grown plants.

In order rapidly to determine changes in the osmotic pressure of various pure sugar solutions used in these experiments, a Zeiss hand sugar refractometer was employed. This instrument has a scale which reads from 0 to 30 per cent dry matter and can be read accurately to 0.1 per cent. It was standardized by use of pure sucrose solutions of known osmotic pressure. A graph showing the actual osmotic pressure at 20°C. to 25°C. of sucrose solutions corresponding to refractometer readings at 28°C. was made. Inasmuch as the refractometer reading varies with temperature, appropriate correction by use of the International Table of Temperature Corrections issued in 1936 for the stand-

ard models of the refractometer were made to convert all readings to 28°C. If the osmotic pressure of the solution were desired at any temperature other than that given on the graph, corrections could be made by use of the data of Morris, Holland, Meyers, Cash, and Zinn found in the International Critical Tables (1936).

Inasmuch as water moves along diffusion pressure gradients, the limits of tension developed in the xylem sap at any one point in the plant could be calculated if the diffusion pressure deficit (DPD) of water in the adjacent living cells could be determined. Pure sucrose solutions of known concentration were injected directly into the hollow spaces in the petioles of squash leaves in these experiments and changes in the concentrations of the solutions were studied. When the injected solution increased or decreased in concentration, the diffusion pressure deficit of water in the tissue lining the petiole cavity was respectively greater or less than that of the test solution. If a rapid penetration of sucrose into the living cells or a diffusion of solutes out of them occurred, the above statement would not be true. The entry of sucrose into the living leaf cells was studied by the half leaf method described below but no determinations were made on possible diffusion of solutes out of the cells.

Injection was accomplished by means of medicine droppers whose tips were drawn out into fine capillaries. Prior to injection two small holes about 1 mm. in diameter were pushed through the petiole between two vascular bundles, entering the air space near either end of the petiole. These holes were covered with vaseline to plug any xylem vessels which might have been injured. This was a necessary precaution, since an injured vessel tended to draw solution into it. Two holes were advisable since injection of liquid into the lower would force air out of the upper, preventing leakage of liquid around the point of injection. The capillary was placed through the lower hole entering the inner space and the desired amount of sugar solution (usually 1 to 2 ml.) of known osmotic pressure was injected directly into the air chamber. The capillary was then removed and both holes sealed with vaseline. Small samples (2 or 3 drops) of solution could be removed from the air chamber at will by inserting the capillary in the lower hole. Changes in the osmotic pressure of the injected solution were then determined by taking a refractometer reading of the sample and referring to the standardization graph.

In the earlier experiments the injected petioles were covered with a thin coat of vaseline to prevent an exchange of gas through the tissue to the inner space. In order that the rate of exchange of gas through the petiole might be determined, a squash leaf with a petiole 20 cm. long was attached to a manometer so that the manometer recorded the air pressure in the petiole. Pressure was applied until a manometer reading of 55 cm. of mercury

was obtained. Air was forced through the leaf and petiole under this pressure at a rate of 30 ml. per hour. During this slow passage, air from the hollow petiole was found to be expelled to the outside chiefly through the lenticels when the leaf was immersed in water. Such a slow rate of gas movement under a pressure drop of 55 cm. of mercury is in contrast to the findings by Glasstone (1942) of very high rates of air movement through plants.

Such a slow rate of penetration of gas through the walls of the petiole indicated a high resistance to gaseous exchange through intercellular spaces. Consequently the vapor pressure of the gas in the hollow petiole should be in approximate equilibrium with the water in the cell walls lining the cavity and would not be directly influenced by the external atmosphere.

Rapid absorption of the sucrose by the living cells resulting in an increase in the osmotic pressure of the xylem sap would constitute a serious error in the method. This would cause the calculated tension to be higher than the actual tension.

To test this possibility, two vigorous squash plants about six feet long, growing in culture solution were selected. The petioles of ten leaves on plant 1 and six leaves on plant 2 were covered with a thin layer of vaseline and every other leaf was injected with approximately 2 ml. of a sucrose solution having an osmotic pressure of 9.3 atmospheres. The injection was done at 11:00 a.m. Immediately the laminae of all the leaves were bisected lengthwise and half of each lamina was removed from the plant, placed in a vial, and immediately frozen for later osmotic pressure measurements. The remaining halves of the leaf blades were left on the plants. At intervals of 3, 6, 22, and 46 hours after injection, the remaining halves of the laminae of two injected and two non-injected leaves were collected and their osmotic pressures determined. This process constitutes a method of comparing the change in osmotic pressure of the expressed sap from the lamina of an injected leaf during an extended time period with the change occurring in a non-injected leaf under otherwise similar conditions. The osmotic

TABLE 1. *The effect of injection of petioles with sugar solutions on the osmotic pressure of leaf sap, July, 1942. Osmotic pressure expressed in atmospheres at 22½°C. Each osmotic pressure measurement was made on half of the leaf lamina.*

Leaf No.	Osmotic pressure of leaf sap			Osmotic pressure of injection solution					
	Initial	Final	Gain or loss	July 22			July 23		July 24
				11:00 a.m.	2:00 p.m.	5:00 p.m.	8:30 a.m.	2:45 p.m.	8:45 a.m.
Plant No. 1									
	July 22 10:30 a.m.	July 22 2:00 p.m.							
1	7.71	8.68 +	0.97
2 ^a	8.23	9.01 +	0.78	9.3	4.8
3	7.48	9.01 +	1.53
4 ^a	7.84	8.36 +	0.52	9.3	5.1
	July 22 10:30 a.m.	July 22 5:00 p.m.							
5	8.03	8.23 +	0.20
6 ^a	8.23	8.49 +	0.26	9.3	5.0	4.5
7	8.49	8.49	0.00
8 ^a	7.57	9.80 +	2.23	9.3	5.2	4.4
	July 22 10:30 a.m.	July 23 9:00 a.m.							
9	8.36	8.49 +	0.13
10 ^a	8.29	8.10 -	0.19	9.3	5.3	4.5	2.5
Plant No. 2									
	July 22 10:30 a.m.	July 23 9:00 a.m.							
1	9.08	7.31 -	1.77
2 ^a	8.16	7.18 -	0.98	9.3	4.5	4.3	1.7
	July 22 10:30 a.m.	July 24 9:00 a.m.							
3	7.51	7.64 +	0.13
4 ^a	8.23	7.18 -	1.05	9.3	4.5	4.4	1.7	...	1.5
5	8.23	7.05 -	1.18
6 ^a	9.34	7.71 -	1.63	9.3	5.1	4.6	2.4	3.5	2.3

^a Petiole injected 11:00 a.m., July 22.

pressures reported for the leaves are values for the expressed sap determined by immediately freezing the tissue, expressing the sap, and determining the osmotic pressure cryoscopically. Currier (1944) has described the semi-microcryoscopic method employed in these determinations.

Table 1 shows that injection of the petioles with sugar solutions did not in this case significantly increase the osmotic pressure of the laminae. The gain in OP by leaf 8, plant 1, deviates significantly from the value found in the other three leaves of this group and this may result from the absorption of sugar; but leaf 3, plant 1, also shows a similar deviation from its group, although the petiole in this case was not injected. Such individual variations in the osmotic pressure of leaf laminae are probably due to differences in exposure of the laminae and the treated leaves do not show consistently higher osmotic pressures as would be expected if the sucrose solutions were rapidly absorbed and transported to the blades.

EXPERIMENTAL RESULTS.—*Diffusion pressure deficits in field-grown squash plants.*—Reference to figure 1 shows the changes in the osmotic pressures of sugar solutions injected into the hollow petioles of Acorn squash plants growing in the field near a small creek. The plants whose stems extended from

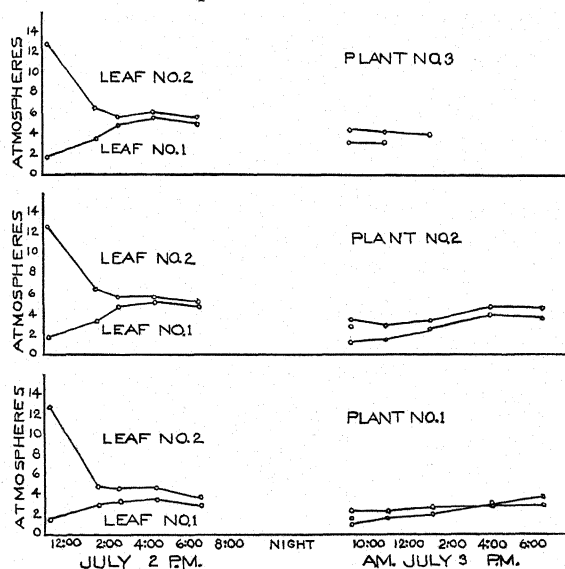


Fig. 1. Changes in the osmotic pressures of sugar solutions injected into the petioles of soil grown Acorn squash.

nine to twelve feet were not watered at any time. Approximately three feet from the tip of each plant two leaves separated by an untreated leaf were selected for the determination. At 11:15 a.m. on July 2 one petiole of each plant was injected with a solution which had an osmotic pressure of 12.3 atmospheres and the other with a solution having an osmotic pressure of 1.8 atmospheres. The petioles were completely covered with vaseline and small samples of solution were taken at the time

intervals indicated in figure 1 for determination of changes in osmotic pressure.

Similar results were obtained on all three plants. Immediately after injection the solutions very rapidly approached each other in concentration. At 2:00 p.m. the lower concentration in plant 1 had increased to 2.8 atmospheres by the diffusion of water into the tissue. At the same time the high concentration had become diluted from 12.3 to 4.5 atmospheres. This indicates that the diffusion pressure deficit of the tissue bathed by these solutions

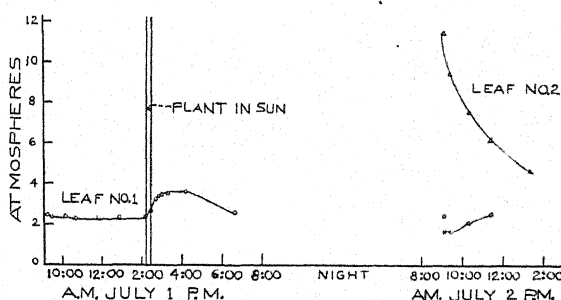


Fig. 2. Changes in the osmotic pressures of sugar solutions injected into the petioles of a Hubbard squash plant growing in culture solution.

was at that time less than 4.5 atmospheres and more than 2.8 atmospheres. Further observation showed a continued increase in concentration of the dilute solution to 3.7 atmospheres at 4:45 p.m. and only a slight drop to 4.4 atmospheres in the more concentrated solution. The DPD of the inner tissue of the petioles must have been somewhere between these two values at 4:45 p.m. At this time it was noted that the plants showed a slight transient wilting of all the leaves. Recovery from this wilting was observed to have occurred at 6:50 p.m. Correlated with this there had been a movement of water out of the cells of both petioles into the solutions, causing them to become diluted to 3.1 and 3.9 atmospheres respectively. This means that the DPD of the tissue had fallen from the higher values previously observed to these, or lower values.

The following morning both solutions, which had become diluted overnight when the DPD of the tissue was undoubtedly low, gradually increased in concentration as the day progressed, reaching a value approximately equal to that of the previous day. Although the DPD of the injected solution probably lags behind the DPD of the tissue when the latter is changing rapidly, the rapid change in concentration noted at the beginning of the experiment indicated that these solutions quickly approached the DPD of the tissue.

Diffusion pressure deficit in culture solution squash.—Figure 2 shows the variation in concentration of sugar solutions injected into the petioles of a five-week-old squash plant growing in culture solution in the greenhouse. Before the treatment the plant had been brought into the laboratory. Three of its petioles were injected at 9:00 a.m. with sugar solution of an osmotic pressure of 2.4 atmos-

pheres. This concentration dropped after injection to 2.2 atmospheres and then rose to 2.3 atmospheres where it remained until 2:10 p.m. During this time the plant had been in the relatively constant environment of the laboratory where the light intensity was low and the humidity was high. As a result of being placed outside in the sun for 20 minutes at 2:10 p.m. the plant, which had been grown under more moderate conditions, began to wilt.

The increased water deficit which resulted in increased tensions on the xylem contents was reflected in the sharp rise in the concentration of the in-

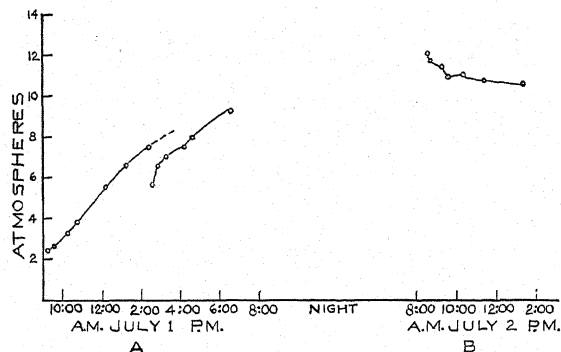


Fig. 3. Changes in the osmotic pressures of sugar solutions injected into the petioles of a Hubbard squash during wilting.

jected solutions from which water moved into the stem. There was an appreciable lag, however, in the concentrating of the injected solutions. A greater increase in concentration of solution in leaf 2 (not shown) resulted, probably from the fact that the volume of solution in this case was smaller than in leaf 1 and equilibrium could be more nearly established between the tissue and the bathing solution.

The solution in leaf 1 increased in concentration after the plant had been brought back into the laboratory until 4:03 p.m. At 3:00 p.m. the leaf was observed to be recovering and the concentration of the solution did not change appreciably until after 4:00 p.m. when it decreased to an osmotic pressure of 2.7 atmospheres at 7:00 p.m.

On the following morning leaf 1 was reinjected with a sugar solution OP 1.8 and leaf 2 with a solution OP 11.5. There was a very rapid drop in concentration of the solution in leaf 2 and only a slow increase in solution 1, indicating a DPD of less than 3 atmospheres.

Diffusion pressure deficit in squash plants during wilting.—Another squash plant growing in culture solution was brought into the laboratory, removed from the solution and allowed to wilt under the moderate conditions of the laboratory. The gradual increase in the osmotic pressures of sugar solutions injected into the petiole of leaf 1 is shown in figure 3. Five hours after the original injection the solution had increased from 2.5 to 7.4 atmospheres osmotic pressure. At this time the solution in the petiole was exhausted and a solution of OP

5.5 was injected into the petiole as shown by the break in the graph (figure 3A). Extrapolation indicates that the original solution was increasing in concentration at a rate that would have caused it to have an osmotic pressure equal to the final OP of the solution injected later had reinjection not been necessary. Ten hours after the wilting had begun the concentration of solutions in leaves 1 and 2 (not shown) indicated diffusion pressure deficits at least as high as 9.2 atmospheres and 8.3 atmospheres respectively. When these leaves were injected the following morning with solutions having a DPD of 12.0 atmospheres (fig. 3B) water moved from the petioles into these solutions which became diluted. Diffusion pressure deficits at least as low as 10.5 atmospheres and 10.8 atmospheres respectively were indicated in leaves 1 and 2 at this time.

Indication that these values approach equilibrium values between the injection solutions and the adjacent tissue is found in the fact that the OP of the injected solution remained almost constant for three hours, which is evidence that the rate of change of tensions in the xylem elements was sufficiently slow at this late stage of wilting to allow the injected solution to come into approximate equilibrium with the neighboring cells.

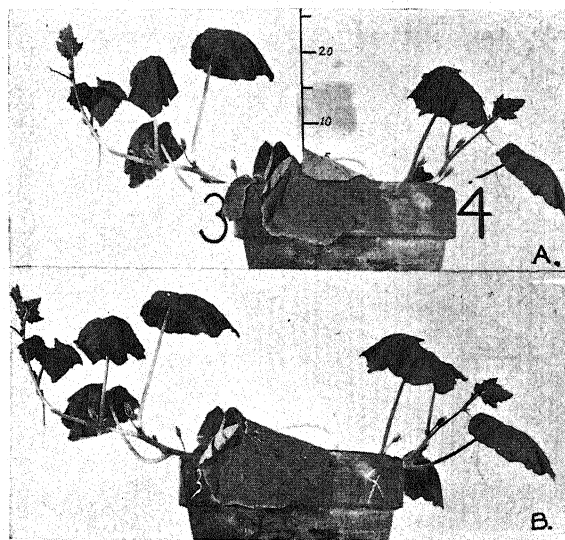


Fig. 4. A, two wilted Hubbard squash plants growing in soil before injection of petioles with sugar solutions; B, five hours after their petioles had been injected with sugar solutions.

In order to avoid the possibility that decreases in concentrations of solutions having high OP and increases in concentrations having low OP were only a reflection of adjustment between the solutions through the plant into which they were injected and not a reflection of the DPD of the plant sap, two young squash plants were injected, one with solutions of low OP only and the other with solutions of high OP.

Figure 4A shows these two young Hubbard squash plants growing in a pot of soil. Water was withheld from the soil and the plants gradually allowed to wilt for five days in the laboratory as shown. At this time seven petioles (numbered from

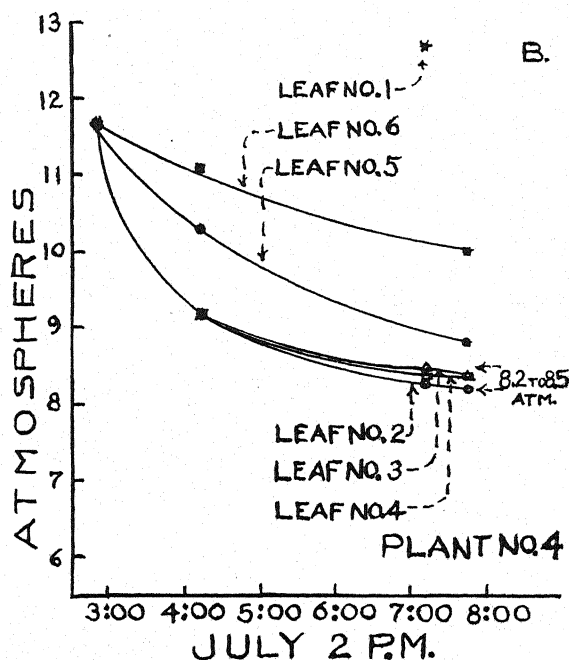
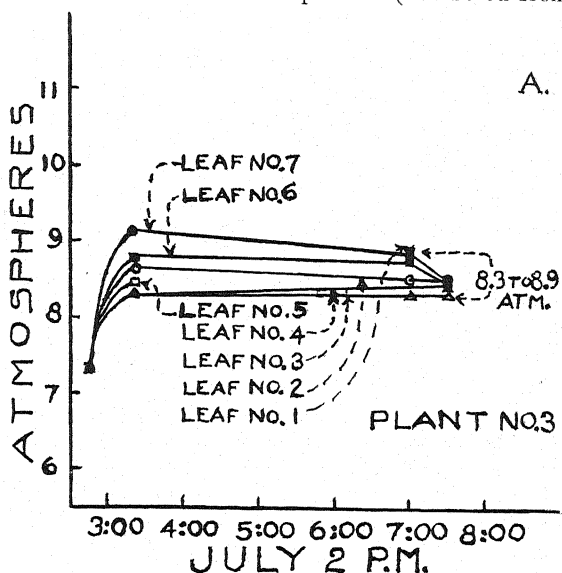


Fig. 5. Changes in the osmotic pressures of sugar solutions injected into soil grown wilted squash plant petioles. A, plant 3; B, plant 4.

tip to base) of plant 3 were injected with a sugar solution having an osmotic pressure of 7.3 atmospheres, and six petioles of plant 4 were injected with a solution having an osmotic pressure of 11.7 atmospheres. Figure 5 shows the changes in the concentration of the solutions with time.

After the solutions had been in the petioles five hours the plants were again photographed and figure 4B shows that the treatment did not visibly change the turgor of the plants. Reference to figure 5A shows that the solutions in plant 3 had come into approximate equilibrium with the tissue of the petioles as the concentration curves had become almost flat. The DPD values represented by these curves fell between 8.3 and 8.9 atmospheres in petioles of plant 3.

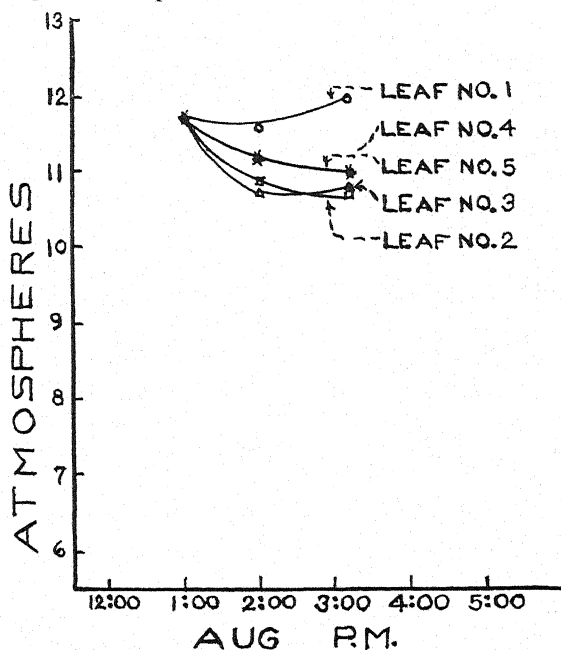


Fig. 6. Changes in the osmotic pressures of sugar solutions injected into the petioles of a soil grown wilted squash.

However, only the solutions injected into leaves 2, 3, and 4 of plant 4 (figure 5B) reached equilibrium with the petiole cells during this interval, indicating values of approximately 8.2 to 8.5 atmospheres. Solutions injected into 5 and 6, which were much more wilted than the upper leaves, decreased uniformly and rapidly, but because of the great difference between the DPD of the cells and of the injection solution, and because of the large volume of solution used for the injections, were unable to gain water fast enough from the cells to approach equilibrium in this time. The solution injected into the tip leaf (leaf 1) increased slightly in concentration to 12.7 atmospheres at which time all of the solution was exhausted by one determination.

Results of a similar experiment on a squash plant which had been in the laboratory for ten days and had slowly wilted are given in figure 6. Diffusion pressure deficits in the xylem of this plant which had slowly wilted were found to be between approximately 11.0 and 10.5 atmospheres (except for the tip) which is somewhat higher than in the previous experiments. These values are of the same

order of magnitude as those reported for the wilted culture solution plant.

DISCUSSION.—If there is a direct water contact from the xylem to the thin walled parenchyma cells lining the cavity in a squash petiole, the diffusion pressure of the water in the xylem immediately adjacent must approach that in the parenchyma particularly when water absorption is slow and transpiration is reduced. That there are continuous water columns in the xylem of even extremely wilted plants was shown by direct observation in connection with this investigation (Stocking, 1943) as well as by other workers (Bode, 1923; Holle, 1915). Although the diffusion pressure of water is a function of the temperature, the osmotic pressure, the turgor pressure or tension, surface forces (capillarity in the walls bordering on air spaces), and any other fields of force which may be acting on the water (adsorptive, imbibitional, electrical, etc.), the primary factor affecting it in the center of tracheary lumina would be the tension in the column if water is moved through the plant in tensile columns as proposed by the cohesion theory.

Let us consider how the diffusion pressure of water in the lumina of non-living tracheary elements is affected by the separate factors mentioned above. Temperature differences are not significant in these experiments.

The concentration of the xylem exudate from a five-week-old squash plant growing in the original culture solution from which the other culture solution plants were taken was determined by collecting the exudation from the stump after the top had been removed at the cotyledons. The exudate was found to have an osmotic pressure of 1.9 atmospheres as determined cryoscopically. Although it is realized that this value is not exact for all of the plants at all times, it indicates the low OP of the tracheary sap and is in general agreement with the low values reported for squash xylem exudations (Crafts, 1936). For purpose of calculation it will be used in this discussion.

In these experiments on prostrate plants there were no significant differences in the gravitational field. The existence of continuous water columns eliminated the possibility of capillarity as a primary factor affecting the diffusion pressure of the sap except indirectly as a component of imbibitional forces associated with the wet walls bordering on air spaces.

Xylem sap is relatively free of colloids but the cellulose walls of the elements must present highly important adsorptive fields. Woodhouse (1933) contended that adsorption can aid in supporting the weight of water "so that any hydrostatic pressure in the tree might be eliminated." The investigation of Stamm and co-workers (1934, 1938) indicates, however, that the forces of adhesion extending out from the walls of water-filled xylem elements have little influence upon the bulk of the water in these elements. It can be safely assumed that similarly in the relatively large-vesselled

squash, forces of imbibition are not of direct importance in determining the diffusion pressure of water in the lumina centers, but only as they may be translated into tensions in this region. Undoubtedly in the hypothetical condition of water equilibrium within an area in a plant, the DPD would be uniform across this region but would be largely a result of different forces depending on the particular tissue, cells and even parts of cells under consideration.

In the vacuoles of living cells, for instance, the DPD would primarily be a function of the OP and turgor of the cells. In the adjacent protoplasmic layer probably imbibitional forces would become more dominant than the OP. This would also be true within the walls, but farther removed from the walls within a dead tracheary element adsorption would have relatively little influence and the DPD would become chiefly a function of the OP and tension or turgor. Consequently maximum tensions would be found during periods of water deficits within the lumen centers.

If we neglect the influence of electrical potentials, we then find that the diffusion pressure of water in the center of the xylem lumina is chiefly affected by the osmotic pressure of the sap, and the tension or turgor present. Consequently a state of subatmospheric pressure or tension will exist when the diffusion pressure is reduced more than can be accounted for on the basis of the solute concentration alone. A state of turgor would exist when the reduction in diffusion pressure is less than that accounted for on the basis of the solute concentration.

Although recent work indicates the possible existence of secretional phenomena in plant tissue, a purely diffusional mechanism of water movement has been assumed in making all calculations in this paper. Tension is used to designate any condition in a water column when the pressure on it is less than atmospheric. Actually an unstable state exists in the column only when the pressure falls below the vapor pressure of the water at that temperature.

Table 2 shows the range in diffusion pressure deficit measurements for sugar solutions injected into the petioles of the experimental plants and also the tensions in the xylem elements calculated by subtracting the factor of 1.9 for the osmotic pressure of the xylem sap from the DPD values. Although it is realized that refinement in technique might modify the values somewhat and result in more specific determinations, it is believed that the magnitudes of possible tensions attained in the xylem of squash petioles under the conditions of the experiments are indicated.

Plants under moderate summer conditions in non-irrigated soil, but growing near a small stream, showed a range in tensions between 1.8 and 4.0 atmospheres during the hot part of the day and at the time of greatest tension showed a slight transient wilt which disappeared about sundown. At this time the tension dropped in the plant and

TABLE 2. Variations in DPD and tensions in the leaves of squash plants growing under various environmental conditions. Tensions calculated by the formula $TP = OP - DPD$. When turgor pressure (TP) becomes negative sub-atmospheric pressure or tension exists and is here listed as a positive tension. $OP = 1.9$ atm.

Plant material	Time		Range in observed		Range in calculated	
			DPD in injected	solution. Atm.	tensions in xylem.	Atm.
Field grown:						
Plant No. 1	2:55 p.m.	4:45 p.m.	3.7	4.4	1.8	2.5
Plant No. 2	2:55 p.m.	4:45 p.m.	4.8	5.8	2.9	3.9
Plant No. 3	2:55 p.m.	4:45 p.m.	5.7	5.9	3.8	4.0
Plant No. 1	9:15 a.m.		0.8	2.1	1.1 ^a	0.2
Plant No. 2	9:15 a.m.		2.1	2.8	0.2	0.9
Plant No. 3	9:15 a.m.		3.2	4.2	1.3	2.3
Culture solution:						
Greenhouse	2:00 p.m.		4.5	5.1	2.6	3.1
	5:00 p.m.		4.3	4.6	2.4	2.7
	8:30 a.m.		1.7	2.5	0.2 ^a	0.6
	2:45 p.m.		3.5	...	1.6	...
	8:45 a.m.		1.5	2.3	0.4 ^a	0.4
Laboratory:						
Subdued light	9:25 a.m.	2:10 p.m.	2.2	2.4	0.3	0.5
Outside:						
In sun			3.6	3.9	1.7	2.0
Wilting plants:						
From culture solution			9.2	10.8	7.3	8.9
Potted in soil:						
Plant No. 1			8.3	8.9	6.4	7.0
Plant No. 2			8.2	8.5	6.3	6.6
Plant No. 3			10.5	11.0	8.6	9.1

^a Turgor pressure atmospheres.

during the night reached values as low as 2.3 atmospheres tension to at least 1.1 atmospheres positive pressure in the xylem. This diurnal fluctuation in diffusion pressure deficits is in general agreement with the known diurnal variation in water balance and osmotic pressures within plants.

Culture solution plants growing in the greenhouse during a warm summer day showed a range in tensions between 2.6 and 3.1 atmospheres during the early afternoon and reached values at least as low as 0.6 atmospheres tension to 0.4 atmospheres pressure during the night. When the culture solution plants were grown in the laboratory under conditions of subdued light the tensions calculated showed a fairly uniform value between 0.3 and 0.5 atmospheres. When placed in the sun a rapid rise was observed correlated with wilting. Recorded values of tensions were in this case only 1.7 to 2.0 atmospheres but the actual values must have been somewhat higher as the conditions of the experiment were such that even approximate equilibrium was not established between the plant tissue and the experimental solution.

When the culture solution plants were removed from the solution and allowed to wilt, the tension in the xylem gradually increased and reached a value of 7.3 to 8.9 atmospheres. Similar plants growing in soil when wilted in the laboratory gradually increased in tensions to 6.3 to 9.1 atmospheres. These

values for wilted squash plants, representing water absorbing powers ranging from 8.2 to 11.0 atmospheres, are interesting when compared with the potential of water at the permanent wilting percentage of approximately 16 atmospheres determined by vapor pressure methods (Veihmeyer, Edlefsen, and Hendrickson, 1943). No explanation is advanced at this time to explain this apparent discrepancy and further experiments should be conducted to clarify this point.

The observation that in two of the wilted plants the tip leaves possessed a higher DPD than the rest of the plant although they remained turgid while the other leaves were wilting also needs further investigation and substantiation. Beck (1928) has suggested that the greater water-absorbing power of young leaves of *Sedum* could be explained on a basis of the lower coefficient of elasticity in the young cell walls than in the older ones resulting in lower wall pressures. This, however, does not appear to be the case in wilted squash plants for the tip leaves are obviously turgid and expanding while the older leaves are flaccid and hence would be expected to have low or negative wall pressures. Beck was unable to demonstrate a gradient of OP between young and old leaves and similar preliminary determinations made during this investigation of the OP of the composite sap expressed from whole squash leaves did not show marked variation be-

tween the young and old leaves. It seems probable that the greater imbibitional pressures of the slightly vacuolated, highly protoplasmic, expanding cells with their thin plastic walls in the young leaves could account for the lowered diffusion pressure of the water in their sap, enabling them to maintain their turgidity in the presence of the wilted lower leaves, or else these cells are actively secreting water into their vacuoles. Kerr and Anderson (1944) have recently come to similar conclusions in regard to the ability of young cotton bolls to absorb water.

SUMMARY

A new method of calculating tensions in the xylem of intact squash plants during normal growth and wilting is described. This method involves the observation by means of a refractometer of changes

in the concentration of pure sugar solutions injected into the hollow petioles of the leaves.

Diurnal fluctuations were observed in the diffusion pressure deficits of the solutions injected into plants growing in the soil and reflected changes in probable tensions of from 4 atmospheres during a warm summer day to positive pressures of more than one atmosphere during the night.

Increasing tensions associated with wilting reached 6.3 to 9.1 atmospheres in squash growing in soil at about the wilting percentage.

The greater water absorbing power of young leaves could not be correlated with the average OP of the sap expressed from the leaves and it is suggested that imbibitional forces may be dominant in this case.

PUCCINELLI PACKING CO.,
TURLOCK, CALIFORNIA

LITERATURE CITED

- ASKENASY, E. 1895. Ueber das Saftsteigen. Bot. Centralbl. 62: 237-238.
- BECK, W. A. 1928. Osmotic pressure, osmotic value, and suction tension. Plant Physiol. 3: 413-440.
- BODE, H. R. 1923. Beiträge zur Dynamik der Wasserbewegung in den Gefäßpflanzen. Jahrb. Wiss. Bot. 62: 92-127.
- BÜHM, J. A. 1892. Ueber einen eigenthümlichen Stammdruck. Ber. Deutsch. Bot. Ges. 10: 539-544.
- CRAFTS, A. S. 1936. Further studies on exudation in cucurbits. Plant Physiol. 11: 63-79.
- . 1939. Solute transport in plants. Science 90: 337-338.
- CURRIER, H. B. 1944. Cryoscopy of small amounts of expressed tissue sap. Plant Physiol. 19: 544-550.
- DIXON, H. H. 1924. The transpiration stream. Univ. Lond. Press Ltd.
- , AND J. JOLY. 1894. On the ascent of sap. Ann. Bot. 8: 468-470.
- EATON, FRANK M. 1941. Water uptake and root growth as influenced by inequalities in the concentration of the substrate. Plant Physiol. 16: 545-564.
- ERNEST, ELIZABETH C. M. 1934. The effect of intercellular pressure on the suction pressure of cells. Ann. Bot. 48: 915-918.
- GLASSTONE, VIOLETTE F. C. 1942. Passage of air through plants and its relation to measurement of respiration and assimilation. Amer. Jour. Bot. 29: 156-159.
- HOLLE, HANS. 1915. Untersuchungen über Welken, Vertrocknen und Wiederstraftwerden. Flora 108: 73-126.
- KERR, T., AND D. B. ANDERSON. 1944. Osmotic quantities in growing cotton bolls. Plant Physiol. 18: 338-349.
- MACDOUGAL, D. T., AND J. B. OVERTON, AND G. B. SMITH. 1929. The hydrostatic-pneumatic system of certain trees; movement of liquids and gases. Carnegie Inst. Wash. pub. 397.
- MEER, B. S. 1938. The water relations of plant cells. Bot. Rev. 4: 531-547.
- NORDHAUSEN, M. 1921. Weitere Beiträge zum Saftsteige-problem. Jahrb. Wiss. Bot. 60: 307-353.
- OPPENHEIMER, H. R. 1930. Kritische Betrachtungen zu den Saugkraftmessungen von Ursprung und Blum. Ber. Deutsch. Bot. Ges. 48: 130-140.
- PEIRCE, G. J. 1936. The state of water in ducts and tracheids. Plant Physiol. 11: 623-628.
- PRESTON, R. D. 1938. The contents of the vessels of *Fraxinus americana* L., with respect to the ascent of sap. Ann. Bot., N. S. 2: 1-22.
- PRIESTLEY, J. H. 1935. Sap ascent in the tree. Sci. Prog. 30: 42-56.
- RENNER, O. 1911. Experimental Beiträge zur Kenntniss der Wasserbewegung. Flora 103: 171-247.
- . 1912. Versuche zur Mechanik der Wasserversorgung. I, Der Druck in den Leitungsbahnen von Freilandpflanzen. Ber. Deutsch. Bot. Ges. 30: 576-580.
- STAMM, A. J., AND L. A. HANSEN. 1938. Surface-bound versus capillary-condensed water in wood. Jour. Physical Chem. 42: 209-214.
- , AND W. K. LAUGHBOROUGH. 1934. Thermodynamics of the swelling of wood. Colloidal Symposium Monograph, 1934: 121-132.
- , AND R. M. SEBRAG. 1934. Adsorption compression on cellulose and wood. I. Colloidal Symposium Monograph, 1934: 133-142.
- STOCKING, C. R. 1943. Continuity, tensions, and redistribution of water in plants. Ph.D. thesis. University of California.
- THUT, HIRAM F. 1932. Demonstrating the lifting power of transpiration. Amer. Jour. Bot. 19: 358-364.
- URSPRUNG, A. 1935. Osmotic quantities of plant cells in given phases. Plant Physiol. 10: 115-133.
- VEILMEYER, F. J., N. E. EDLEFSEN, AND A. H. HENDRICKSON. 1943. Use of tensiometers in measuring availability of water to plants. Plant Physiol. 18: 66-78.
- WARNE, L. G. 1942. The supply of water to transpiring leaves. Amer. Jour. Bot. 29: 875-884.
- WOODHOUSE, EDWIN D. 1933. Sap hydraulics. Plant Physiol. 8: 177-202.

ANATOMY OF CRYPTOSTEGIA GRANDIFLORA WITH SPECIAL REFERENCE TO THE LATEX SYSTEM¹

H. Weston Blaser

THE ANATOMY of latex-bearing plants has received much attention since the early days of the study of plant structure; recently, as a result of the search for new sources of rubber, a modern re-investigation has been initiated. *Cryptostegia grandiflora* (Roxb.) R. Br. is receiving special attention since it may develop as a usable source of good-quality rubber. Technical difficulties of extraction, at present, limit the amount of rubber obtained to that which is bled from the stems. Experimental and practical problems as well as attempts to reduce the tedium of extraction methods are likely to involve structural details obtained from a general anatomical study.

Literature on laticiferous cells and tubes is voluminous and anatomical details have been described for many families. David (1872) describes and characterizes several kinds of laticiferous ducts²: intercellular canals, multicellular vessels and unicellular tubes. de Bary (1877) classifies the latter two types as articulated and non-articulated ducts. Tschirch (1906) presents a review covering the earlier literature and Sperlich (1939) brings the general data up to date. With these general reviews readily available, it is unnecessary to present a lengthy statement here.

Solereder (1908) briefly summarizes a large part of the literature concerning the family Asclepiadaceae, to which *Cryptostegia* belongs. The general anatomy of the family varies widely but probably all species contain laticiferous tubes of the non-articulated type. The time of origin of the tubes varies with the species as is shown by Schmalhausen (1877), Chaveaud (1891) and Schaffstein (1932). Distribution and duration of the tubes also vary. Some species are said to contain both primary and secondary tubes (of primary and secondary tissues) although the majority seem to contain only primary ones. Accessory latex cells (parenchyma cells containing latex) are reported in some species. No statements dealing specifically with *Cryptostegia* have come to the author's attention. Since the anatomical treatment of latex tubes is sketchy in the literature, the details of anatomy of this species may be of value in coordinating isolated statements concerning the structure of latex tubes in general.

MATERIALS AND METHODS.—Materials were obtained from three sources. Seeds, obtained from

Haiti, were germinated and plants grown in the greenhouse and field at Cornell University. Preserved material of plants, two years old, was obtained from the station of the U. S. Department of Agriculture at Coconut Grove, Florida. Large plants from the U. S. D. A. station at Beltsville, Maryland, were transplanted to the Cornell greenhouse. For embryo studies seeds soaked for twenty-four hours were imbedded intact or embryos were removed and imbedded. All materials were fixed in F. A. A. and sections were made from unimbedded material and from celloidin- or paraffin-imbedded portions. Heidenhain's iron-alum-haematoxylin with or without a counterstain of safranin or Bismark brown proved the most important stain. Photomicrographs were made by W. R. Fisher.

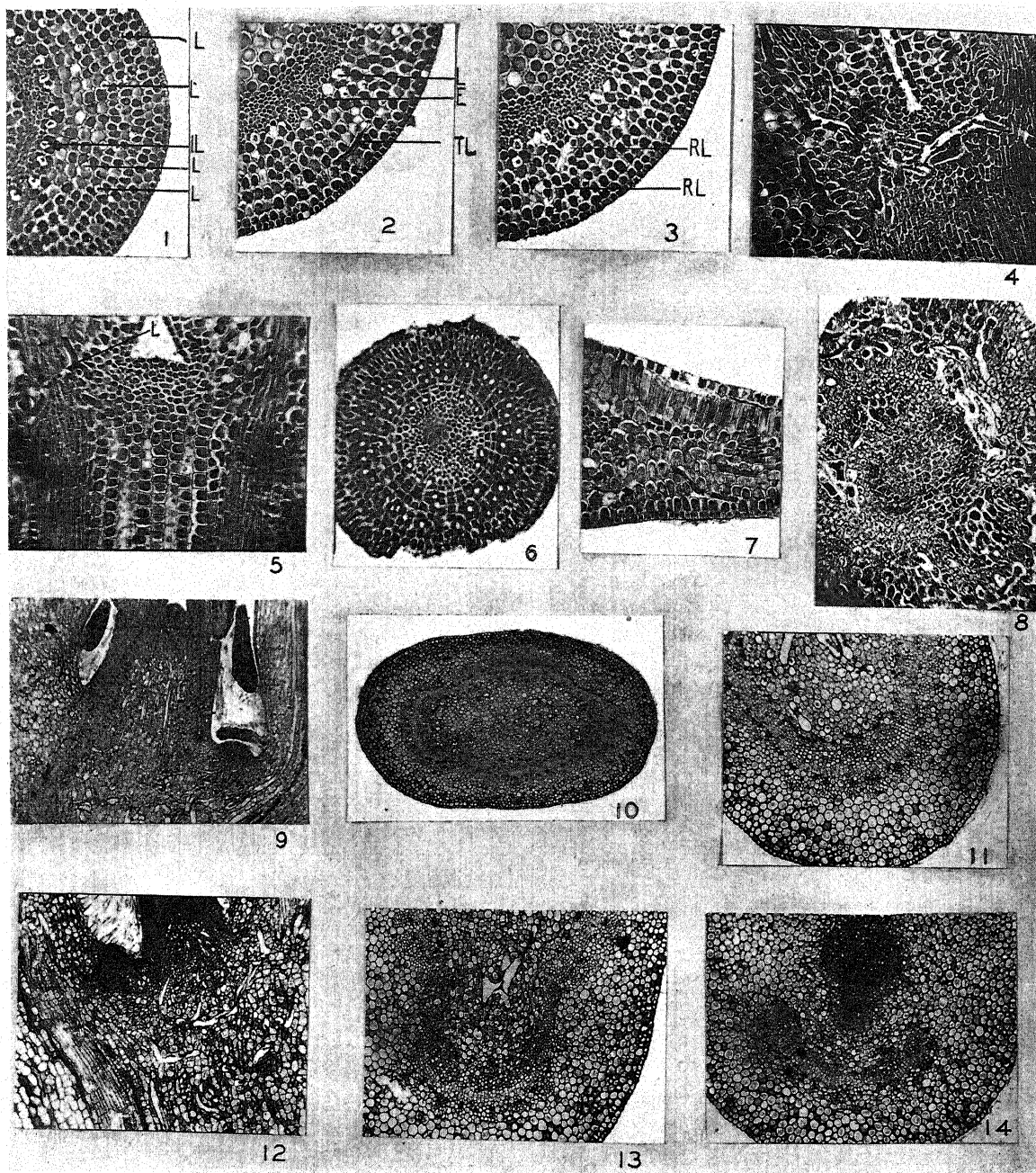
EMBRYO AND SEEDLING.—Chaveaud (1891) and others have shown that latex tubes originate in some species in the embryo, while in others they differentiate after germination. In *Cryptostegia* embryos, the latex tubes are well developed and have already formed a branching system when the seed is mature. In the hypocotyl, several concentric bands of tubes occur between the tangential rows of cortical cells (fig. 1). The tubes of the innermost ring are of larger diameter than the others. Anatomical evidence suggests that the inner cycle represents the basic ring of latex tubes and that all others are branches. For conclusive evidence, studies of embryonic development are necessary. There are many radial connections (fig. 3) and only the inner ring is in a uniform position. The tubes branch tangentially also (fig. 2) but less frequently. They extend nearly to the tip of the radicle (fig. 6) and into the cotyledons (fig. 4, 5, 7, 8). The plumule is rudimentary in the embryo (fig. 5) and epicotyledonary tubes can be found only after germination. At the cotyledonary node, the tubes branch freely and supply the cotyledons with a considerable number of latex tubes. Some of the branches extend through the gap and enter the plumule, or pass outward on the upper side of the cotyledonary trace.

It is impossible to describe or illustrate fully the complexity of tube-branching at the node, but it can be partially shown in selected sections (fig. 4, 5, 8). A transverse section at the node close to the region of separation of the cotyledons from the axis (fig. 8) shows the condition of the tubes as they branch and pass around the traces of the cotyledons. The central region of the stem apex is visible between the two traces at the upper left and lower right of the figure. A nearly median longitudinal section (fig. 5) shows, on the left, a tube with two branches; one of these extends into the stem apex and the other passes along the upper side of the cotyledonary bundle. A section near the

¹ Received for publication November 17, 1944.

Researches on anatomy of latex-bearing plants supported in part by funds from the Rubber Reserve Corporation.

² The terminology used in this paper for the latex-bearing structures is as follows: (a) latex cells, parenchyma cells containing latex, (b) ducts, elongate latex-containing structures which may be (c) canals, intercellular secretory chambers, (d) tubes, elongate unicellular laticiferous ducts or (e) vessels, elongate multicellular laticiferous ducts.



Figs. 1-14.—Figs. 1-8. Anatomy of the embryo of *Cryptostegia*.—Fig. 1. Hypocotyl showing distribution of latex tubes through the cortex. The inner series of larger cells (IL) are probably the basic tubes and others (L) are branches.—Fig. 2. Tangentially branched latex tube (TL) in the same specimen. The small clusters of slender cells among the inner ring of latex tubes are young pericyclic fibers (E).—Fig. 3. Hypocotyl of germinated embryo showing several radial connections (RL) between latex tubes.—Fig. 4. Off-median section near edge of traces to show latex tubes as they enter the apex from the sides of the cotyledons. In all figures, no latex tubes occur in the pith.—Fig. 5. Nearly median longitudinal section showing rudimentary plumule. At the left (L) is shown a tube with branches for the upper side of the cotyledon and the continuing apex.—Fig. 6. Root, near the tip showing the latex tubes. The outer two or three rows of cells are root-cap cells. This section is 180 microns from the tip of the root cap; some latex tubes are present within 90 microns of the tip.—Fig. 7. Cotyledons, showing latex tubes in the mesophyll. On the upper side a subepidermal tube extends nearly the full length of the figure. Two additional tubes in the spongy parenchyma are prominent.—Fig. 8. Transverse section of cotyledonary node. The traces to the cotyledons are toward the upper left and lower right of the figure. The complex nodal branching of the tubes and the entrance of the tubes through the gap are evident.—Fig. 9. Median longitudinal section of the stem apex including three

edge of the traces (fig. 4) shows more of the tubes in the nodal region. In both longitudinal figures the absence of latex tubes in the pith of the hypocotyl is evident. There are no basipetal extensions of the tubes into this region in later development but in the epicotyl there are both cortical and medullary tubes. In the cotyledons the tubes ramify through the mesophyll and extend to the epidermis (fig. 7).

In the internodes of the seedlings, the latex tubes are distributed throughout the pith and cortex. At each node, extensive branching of all tubes results in a condition similar to that of the cotyledonary node. Tubes pass through the gap in both directions and branching provides tubes for the leaves, axillary buds and the continuing apex. The complex anatomy of the nodes is made even more complex by the presence of many branching tubes in the gaps. Forking of the tubes occurs also in the internodes but is less frequent.

The original latex tubes of the embryo by successive branchings, most frequent at the gap regions, form an "elaborate forking system" with no observed fusions. In the absence of fusions, the terms "mesh-work" and "network" of tubes are inappropriate.

THE STEM TIPS.—Apices of stems from the three sources show no essential difference. There is no increase in diameter of the stem apex with age of the plant and axillary buds are essentially like the tips of elongate stems. The nodes successively repeat the complex pattern of the seedling plants. The latex system of the apex (fig. 9) is more mature in the pith, a condition coordinate with the earlier maturation of the pith parenchyma. Three nodes are illustrated in figure 9; the second node is indicated by the numerous transverse sections of tubes which enter a pair of leaves at right angles to the plane of section. The tips of the tubes extend to within a few cells of the surface of the apical meristems. The shortness of the internodes near the apex makes it possible to trace the tubes more readily than in older stems. Elongation of the axis extends the tubes and stretches the regions of branching so that in older stems the continuity of the system is much less striking.

THE NODES.—The tubes which enter the leaves and branches at a node are not restricted to sectors of the stem (fig. 9, 10, 11). Opposite leaves and their axillary buds contain only locally independent

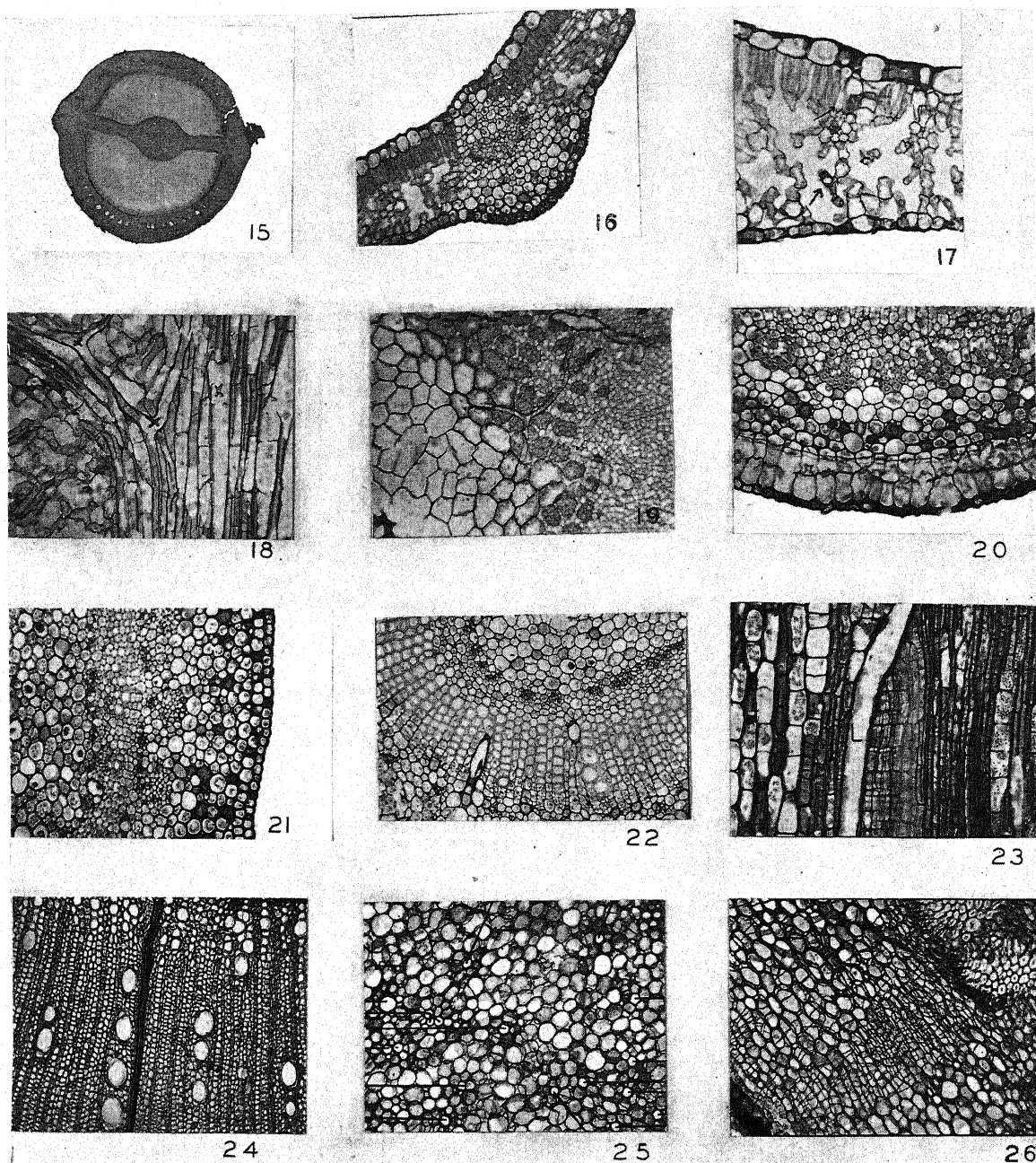
branches of the latex system. Leaves of two succeeding nodes were definitely found to contain branch tubes from the same cauline tube. It is possible that even opposite leaves may contain branches of the same subnodal tube although no such case was observed with certainty. In any case, the system of one portion of the plant is closely associated with that of the rest of the plant. A leaf and its axillary bud also contain connected tubes (fig. 12, 13, 14).

Axillary buds are identical with other actively growing stem tips in their latex systems. The axillary meristem reaches full size very near the tip of the axis (fig. 9) and in elongating produces the first pair of leaves laterally with its accompanying latex tubes (fig. 12). Superposed buds (fig. 12, 14) are found to be branch buds of the main axillary bud and their latex systems are connected. The nodal structure of the latex system (fig. 12, 13, 14) thus involves a multiplication of branch tubes some of which enter the lateral structures while the rest remain as the cauline system.

THE LATEX SYSTEM AND PRIMARY TISSUES.—The latex tubes of the embryo were shown above as structures outside the vascular system. The basic ring is near the patches of young pericyclic fibers, and branch tubes occur throughout the cortex. Further development and branching tend to mask this fundamental origin. Near the apex where growth of the tubes is most active, the cortical tubes extend nearer the tip than do those of the pith. This condition is probably related to the formation of lateral primordia of leaves and buds. Patches of pericyclic fibers differentiate inside of or among the cortical tubes. Procambial strands and young fibers differentiate from the meristem in advance of the growth of latex tubes. A latex tube is never found enclosed in pericyclic fibers and since all the vascular tissues do not differentiate from the meristem simultaneously, the radial branches of latex tubes pass between patches of fibers and through the developing vascular cylinder. Phloem and xylem contain no cells which can be considered phloem or xylem latex-tubes but contain branches of the latex system which may penetrate for short distances. The manner of their origin and development would preclude designation as part of the vascular tissues.

The primary vascular cylinder is a narrow amphiphloic siphonostele, with single large leaf traces

nodes. The lowest node shows young axillary bud at the right. The branching complex of latex tubes of the subtending leaf and bud is evident in part. The diameter of the bud meristem is as great as the apical meristem. The second node is shown only by the nodal complex of latex tubes. The leaves at this node are at right angles to the plane section and many of the latex tubes are cut transversely. The tubes near the tip are of small diameter but the general structure can be seen. Large glandular hairs and slender elongate hairs are present on the ventral surface of the lowest pair of leaves.—Fig. 10. Stem near the fourth node showing the amphiphloic siphonostele, the opposite large leaf traces and the associated branch traces. The medullary latex tubes are branching extensively throughout the pith.—Fig. 11. A section at a higher level than figure 10 showing greater detail.—Fig. 12. Longitudinal section of node showing trace to leaf at left and the superposed axillary buds. The principal bud shows latex tubes of the first pair of leaves which are attached at right angles to the plane of section.—Fig. 13. Interconnected tubes of leaf and bud. The apparently H-formed structure is derived by three successive dichotomies within the same section. The basipetal continuation is a single tube attached at the cross bar of the H. The two branches toward the leaf trace enter the leaf. The two inner ones enter the axillary bud.—Fig. 14. Transverse section at node similar to figure 12 showing superposed buds.



Figs. 15-26.—Fig. 15. Transverse section of two-year-old stem, showing open gaps. Several latex tubes imbedded in the secondary xylem appear as faint dark streaks.—Fig. 16. Mid vein region of young leaf showing latex tubes on both sides of the vein.—Fig. 17. Transverse section of mature leaf showing latex tube in spongy mesophyll, another at the base of the small bundle and a third one extending from the bundle through the palisade parenchyma and turned at right angles just under the epidermis.—Fig. 18. Paralongitudinal section of main vein and side vein showing branching of latex tubes (X).—Fig. 19. Paralongitudinal section of mature leaf showing latex tube in palisade parenchyma and extending under the epidermis in two branches.—Fig. 20. Stem from sixth internode showing periderm which originates in the subepidermal cells. The fibers have partially matured although not all of them have developed thick walls.—Fig. 21. Stem showing patches of young pericyclic fibers. The protophloem clusters of sieve tubes may or may not lie on the same radius as the fibers. Parenchyma cells lie between the fibers and the small sieve tubes.—Fig. 22. Latex tubes imbedded in secondary xylem of young stem.—Fig. 23. Radial section showing latex tube crossing the stele.—Fig. 24. Latex tube imbedded and lengthened transversely in secondary xylem. The rays are uniseriate except the one which includes the latex tube.—Fig. 25. Portion of the pith of a two-year-old stem showing starch-filled parenchyma in close association with the latex tubes. Lines indicate latex tubes. Intermediate parenchyma cells are empty.—Fig. 26. Pericyclic fibers cortex and periderm of two-year-old stem.

to the decussate leaves. Branch traces arise from the cylinder at the side of the leaf gap. The position of traces and gaps is shown in figure 10. The primary xylem forms a rather uniform cylinder with cells radially arranged. The protophloem consists of patches of minute sieve tubes and large parenchyma cells. Some sieve tube areas may be on the same radius as the fiber patches but others lie between them (fig. 21). The metaphloem consists of larger sieve tubes and abundant parenchyma.

The latex system of the leaves is associated with the veins; tubes are present on both surfaces of the bundles (fig. 16). At points of vein branching, the latex tubes fork with the veins (fig. 18). Branches of the tubes extend into the mesophyll freely (fig. 17) and come in contact with the epidermis (fig. 17, 19).

SECONDARY GROWTH.—In the fifth or sixth internode from the apex, a phellogen arises from the subepidermal row of cortical cells. Since no latex tubes are found next to the epidermis, there is no effect upon the latex system by periderm formation (fig. 20). This first periderm persists for at least two years forming rather few cells (fig. 15, 26). When and if a second periderm forms can be determined only from plants older than were available to the author.

Secondary vascular tissues are normal in formation and structure. The leaf gaps remain open for two years, long after leaf abscission (fig. 15). The latex tubes which cross the stele or are gradually enveloped by the partial closing of the gaps are not cut off or broken by secondary thickening but are enclosed or imbedded in the secondary xylem and phloem. In young stems the oblique course of the tubes is retained (fig. 23), and tubes appear in cross sections (fig. 22) as if they were part of the secondary cylinder. With greater enlargement, the latex tubes are enclosed in vascular rays and are nearly transverse (fig. 24). Most rays are uniseriate (fig. 24, 30) but rays containing these latex tubes are three or four cells wide (fig. 31, 32). Thin sections appear broken along the tubes and only thick sections enable observation of the length and path of the tubes. Drawings made with the aid of a camera lucida (fig. 27–33) show optical sections not obtainable by photography. The body of secondary vascular tissues is then seen to contain extended latex tubes but none of the tubes are derived from the cambium. That they remain fully distended and active for at least two years may be related to wall structure and to contact with ray cells and parenchyma of the pith and cortex.

In the pith of old stems, the latex tubes lie within a mantle of a starch-filled parenchyma. The starch-containing cells form a pattern alternating with the empty parenchyma not associated with the latex system (fig. 25).

The latex tubes of the cortex are fully distended but not distorted by secondary growth of the vascular tissues. It would appear that the latex tubes

may be stretched in the cortex just as they elongate in the stele. Associated with the permanence of the latex tubes is the permanence of the cortex itself. Enlargement for two years would be expected to entail some adjustment in arrangement or size of the cortical cells. The cortex of *Cryptostegia* shows clearly that cell division enables the cortex to per-

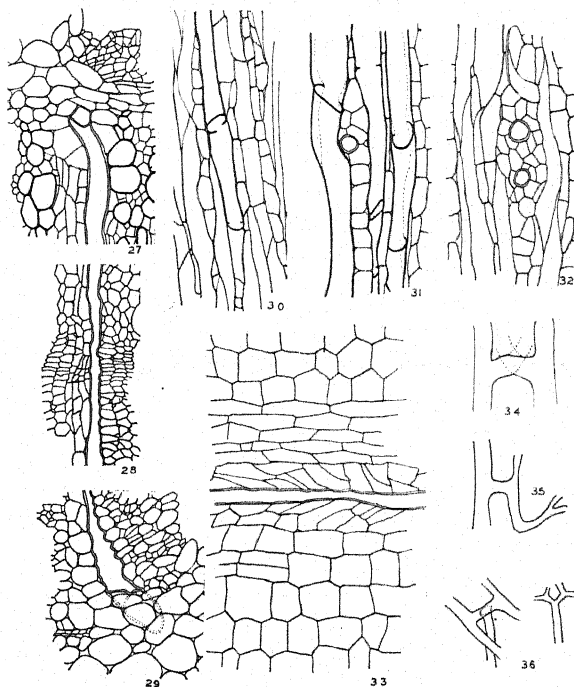


Fig. 27–36.—Fig. 27–29. Portions of three latex tubes in the secondary tissues: (fig. 27) in the xylem near the pith (fig. 28), through the cambium region (fig. 29), in the outer phloem.—Fig. 30. Tangential section of xylem showing vessels, parenchyma and uniseriate rays.—Fig. 31. Tangential section of xylem showing ray with one latex tube included.—Fig. 32. Tangential section of xylem showing ray with two latex tubes included.—Fig. 33. Radial section showing latex tube and ray cells. The latex tubes in figures 27–33 are shown with a double line only for purposes of the drawing and the lines do not indicate wall-thickness.—Fig. 34–36. Reconstruction of apparent H-formed tubes (explanation in text).

sist as a wide unstretched unflattened region. Many strictly radial walls are found and clusters of cells retaining the shape of their mother cells are evident (fig. 26). The cortex seems to be composed of alternating radial bands which differ in the number of cell divisions or in the rate of increase in cell size. It will be noted that there is an increase in width of cortex as well as in tangential enlargement (fig. 21, 26).

DISCUSSION.—The laticiferous system of *Cryptostegia* is composed of a limited number of primary cells which arise early in the ontogeny of the embryo. By acropetal elongation and limitless branching, an elaborate forking system is produced. The tips of branches grow between meristematic cells

close to the apex. The direction and degree of branching seems to be controlled largely by nodal differentiation and the rate of tissue differentiation.

Evidence is as follows:

(a) Nodal complexes of latex tubes are differentiated up to the youngest leaf primordia. The gaps are the regions of greatest tube branching.

(b) Trans-stelar branching is fairly frequent but much less elaborate than gap crossing. The zones of slower differentiation of procambium provide regions for the crossings which do occur.

(c) Early elongation and organization of clusters of pericyclic fibers control the path of these tubes in a similar way.

(d) Latex tubes of the leaf primordia are only slightly shorter than the procambial strands. Elaboration of the branching system in the mesophyll occurs early in the development of the leaf.

There is no evidence of fusion of adjacent tubes nor is there any evidence that new latex cells arise in apical or lateral meristems. It is probable that some parenchyma cells function as accessory laticiferous elements but they are not anatomically specialized.

The method of origin and development of latex ducts has long been recognized as the basis for two general forms—the “H” form and “Y” form. The “H” form is frequently considered as evidence of cell fusion and confluence of cell contents. On the other hand, unarticulated latex tubes which elongate and branch as their axis elongates are considered to be “Y” formed, the arms of the Y suggesting a dichotomy in the direction of growth. Occasional disagreements in the literature may have resulted from the difficulty of observing the much-branched, almost endless cells of the unarticulated type. Sectioned tubes lying adjacent to each other have been found to show no evidence of fusion. However, several sections show apparent “H” form tubes which at first seem to indicate fusion. On close examination of adjacent sections, all of these apparent “H” fusions are found to result from plane of section and path of latex tubes; none of them is a region of cell fusion. Several examples will explain more fully:

(1) The simplest case is the crossing of right and left arms of two “Y” tubes near the walls separating them. The two tubes appear as one except on close examination and comparison with adjacent sections (fig. 34). The upper dotted portions of the tubes in figure 34 were determined from adjacent sections.

(2) The direction of growth of the tubes may not be uniformly acropetal or if it is acropetal, the different growth of surrounding cells distorts the original form (fig. 36). The diagram represents a reconstruction of a tube near a node.

(3) Successive branches very close to each other and in more than one plane may produce a complicated situation which can be traced with difficulty. The accompanying sketches (fig. 36) show a reconstruction of a portion observed.

In transverse section an “H” tube was seen with no evidence at first of any explanation other than fusion (fig. 13). Two arms of the H entered the leaf and two entered the bud. However, basipetally the “H” was connected to a cortical tube at the center of the cross-bar. The cross-bar represented the first of three dichotomies occurring in the same section.

The patches of fiber cells (fig. 2, 3, 20, 26) are designated here as pericyclic fibers. The recent statements of Esau (1938, 1943) regarding the fibers of phloem in *Nicotiana* and *Linum* would indicate that in some plants there is no recognizable pericycle. The evanescent sieve tubes of the earliest protophloem may disappear so quickly that the associated phloem fibers appear to be a distinct pericycle. The “pericyclic region” of *Cryptostegia* may therefore be fibers of the protophloem. In the hypocotyl of the embryo, young fiber patches are distinguishable as small clusters of slender cells (fig. 3). In the older seedlings, no additional fibers are developed in the hypocotyl but larger patches of fibers (15–30 cells in each cluster) are adjacent to definable phloem in the stems (fig. 20, 21, 26). For these fiber patches the author uses the term “pericyclic,” the adjectival form permitting a range of theoretical interpretations but restricting the location of the structures in practical anatomical description. Esau (1943) and Foster (1942), however, finding no “true” pericycle in *Linum* and *Nicotiana* note that the pericycle should be reinvestigated and suggest use of the term “bast fiber” for all extra-xylary fibers. While the need for a comparative concept of “pericycle” is emphasized by Esau’s findings, the suggested change seems highly undesirable. The background for the term “pericycle” is much broader than the present bases for negating its existence. The substitution of a term, which already has many different meanings, for all cortical, medullary, pericyclic and phloem fibers only adds to the confusion. If it is desirable to obviate the difficulty of using the term “pericycle” for a region which is obviously variable in higher plant stems, the adjectival form can be readily substituted with much less confusion. The term “pericyclic region” is a useful and descriptive term. It designates a position and does not preclude the possibilities that the region may be in part or wholly phloem or cortex. If, upon completion of an adequate re-investigation of the “pericycle,” the term is untenable, it can be dropped for better reason than is at present available. The “pericyclic region” is of topographic importance and is as necessary as the term “transition zone,” “cambium region,” “hypodermal region.” It is as definite as many terms which point out regions of transition or transitional cell types. Often such terms are of greater descriptive value than those of rigid definition.

The cytological basis for tremendous cell elongation of the latex tubes presents a problem of interest and importance. That early elongation is in conjunc-

tion with axial growth seems more comprehensible than the persistent elongation and imbedding of tubes in the secondary tissues. Frey-Wyssling (1926, 1933) suggests the possibility that latex-cell walls are elastic and since they are composed of anisotropic material, changes in size occur with changes in turgor. The structural nature of the wall might also permit the elongation of the cell by the method of intussusception. The phenomenon of these latex tubes may explain presence of "secondary latex tubes in rays of the wood" in other species of the family. Woodworth (1932) describes structurally similar tubes in *Beaumontia grandiflora*.

The secondary enlargement of the cortex (fig. 26) by renewed or continued cell division is probably a result of cell division similar to those described by Sinnott and Bloch (1941) for other vacuolated cells. This long-continued cell division emphasizes the dynamic nature of differentiation of the apical meristem. The retention of meristematic activity in restricted regions has frequently led to a loose application of the term "meristem." In these "modern" uses of the term, the "meristem" extends to all regions where noticeably active cell division occurs. It could indeed be stretched sufficiently to include the whole cortex of *Cryptostegia*. The author recommends a re-evaluation of the "modern" uses of the term "meristem" and the greater use of the term "meristematic." Many regions remain or become "meristematic" for varying lengths of time. A "meristem" is a relatively permanent region of cell initiation. Where differentiation has progressed to even a small degree, other terms are applicable; young tissues and old tissues demonstrate varying degrees of *meristematic activity*.

SUMMARY

Latex tubes of the unarticulated type form a branching system in the embryo of *Cryptostegia*.

The tubes are extra-vascular and enter the pith above the cotyledons by way of the cotyledonary gap.

In the young stems latex tubes cross the stele frequently but most of the connections between cortex and pith occur at the gap regions. A single large leaf trace and the branch traces form a common gap which remains open for at least two years. The tips of the latex tubes extend close to the surface of apical and axillary meristems which are identical in size and structure.

The leaves, of mesophytic type, contain latex tubes on both surfaces of the netted veins. Branches occur in conjunction with vein-branching; other branch tubes extend into the mesophyll in all directions. Some reach the epidermis and extend some distance along the inner surface of the epidermal cells.

A periderm arises in the subepidermal layer of the fifth or sixth internode. This first periderm persists for at least two years forming rather few cells.

Secondary growth of the vascular cylinder imbeds the primary latex tubes which cross the stele. No secondary tubes are formed. Growth for two years is accompanied by the elongation of the original latex tubes. The cortex maintains itself by continued cell division of the parenchyma cells. This meristematic activity invites a re-evaluation of some of the "modern" uses of the term meristem.

Pericyclic fibers which are external to the phloem and not associated with the sieve tubes bring attention to recent suggestions that all extra-xylary fibers be called "bast-fibers." The author objects to the latter term and suggests that "pericyclic region" is of anatomical value where a question of validity of the term "pericycle" exists.

DEPARTMENT OF BOTANY,
CORNELL UNIVERSITY,
ITHACA, N. Y.

LITERATURE CITED

- DE BARY, A. 1877. Vergleichende Anatomie der Vegetationsorgane der Phanerogamen und Farne. Leipzig.
- CHAVEAUD, M. G. 1891. Recherches embryologiques sur l'appareil, laticifère des Euphorbiacées Urticacées, Apocynées et Asclepiadées. Ann. Sci. Nat.-Ser. 7 Bot. 14: 1-161.
- DAVID, G. 1872. Über die Milchzellen der Euphorbiaceen, Moreen, Apocynen, und Asclepiadeen. Dissertation. Breslau.
- ESAU, K. 1938. Ontogeny and structure of the phloem in tobacco. Hilgardia 11: 343-423.
- . 1943. Vascular differentiation in the vegetative shoot of *Linum* III. The origin of bast fibers. Amer. Jour. Bot. 30: 579-585.
- FOSTER, A. S. 1942. Practical plant anatomy. Van Nostrand, New York.
- FREY, A. 1926. Die submikroskopische Struktur der Zellenmembranen. Eine polarisationsoptische Methode zum Nachweis der Richtigkeit der Mizellartheorie. Jahrb. Wiss. Bot. 65: 195-223.
- FREY-WYSSLING, A. 1933. Saftergüsse aus turgeszenten Kapillären. Ber. Schweiz. Bot. Ges. 42: 254-283.
- SCHAFFSTEIN, G. 1932. Untersuchungen an ungegliederten Milchröhren. Beih. Bot. Centralbl. 49: 197-220.
- SCHMALHAUSEN, J. 1877. Beiträge zur Kenntnis der Milchsaftgefäße der Pflanzen. Mem. Acad. Imp. St. Petersburg Ser. 7. 24: No. 2.
- SINNOTT, E. W., AND R. W. BLOCH. 1941. Division in vacuolate plant cells. Amer. Jour. Bot. 28: 225-232.
- SOLEREDER, H. 1908. Systematic anatomy of the dicotyledons. Eng. Trans. Oxford.
- SPERLICH, A. 1939. Das trophische Parenchym. B. Excretionsgewebe. Handb. Pflanzenanatomie Bd IV, 1 Abt., 2 Teil.
- TSCHIRCH, A. 1906. Die Harze und die Harzbehälter mit Einschluss der Milchsäfte. 2. Aufl. Leipzig.
- WOODWORTH, R. H. 1932. Diaxylary laticiferous cells of *Beaumontia grandiflora*. Jour. Arnold Arboretum 13: 35-36.

NATURAL BREEDING STRUCTURE IN THE *BROMUS CARINATUS* COMPLEX AS DETERMINED BY POPULATION ANALYSES¹

Jack R. Harlan²

THE SECTION *Ceratochloa* of the genus *Bromus* includes a number of closely related forms or species that present a difficult taxonomic problem (Hitchcock, 1935, and Shear, 1900). This group of forms or species may be tentatively referred to as the *Bromus carinatus* complex. In a study of the genetical variation in this complex conducted at the University of California at Berkeley, some of the fundamental reasons for the taxonomic difficulties were investigated. It was found that the variation pattern included swarms of local races inhabiting a given geographical or ecological province. Often a number of races were found to be growing together in the same field with but few intermediates and little evidence of interbreeding.

Members of the *B. carinatus* complex were shown to have the habit of facultative cleistogamy, reported and described by Harlan (1945). Individual plants usually produce both cleistogamous and chasmogamous panicles. The cleistogamously produced seeds are obligatorily self-pollinated, while the chasmogamously produced seeds are either cross-pollinated or self-pollinated. The natural breeding structure in the complex was investigated by population analyses in an attempt to determine the effect of the facultatively cleistogamous habit on the breeding structure and on the variation exhibited within the species. The results of this investigation are reported in this paper.

MATERIALS AND METHODS.—The general procedure used in making population analyses of this species is outlined as follows:

Collections in the field were made by taking seeds from a single panicle of an individual plant to eliminate the possibility of obtaining seed from two or more plants grown together.

Approximately 35 plants of each collection were spaced in separate nursery rows from seedlings started in flats. The progeny of an individual wild plant is referred to as a family.

The families were classified according to segregation for numerous growth characters and also compared individually.

In a few cases seed was taken from each of two or more plants of a family and a series of second generation families was grown and similarly compared.

Four different groups of families were used and analyzed in this study. Each group was collected in

¹ Received for publication May 5, 1944.

Contribution from the Department of Genetics, University of California, and completed while the author was employed by the Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration. U. S. Department of Agriculture.

² Formerly Research Assistant, University of California, now (1945) Associate Agronomist, Division of Forage Crops and Diseases.

order to study a particular phase of the variation pattern of this species and the possible breeding structure exhibited by it. These groups of families were:

1. A group of 60 families of one particular race, arbitrarily called strain No. 2 was collected for the purpose of investigating variation within a local race. This race was selected because of certain distinctive characters which permit identification in the field. The sheaths are white and hairy, the base of the culms green without anthocyanin pigment, and the leaf blades hairless to moderately hairy and stiffly erect, compared with other local races. The spikelets are green or of a distinctive shade of purple and are also recognizable by virtue of a distinctive color pattern produced by white, papery margins of the lemma.

This race is found abundantly on the plain between the Berkeley Hills and San Francisco Bay and is also recorded from Sonoma County by Dr. G. L. Stebbins, Jr. It appears to be more weedy than other local races occurring in abandoned fields, roadsides, vacant lots and other disturbed areas. The 60 families were collected at 19 stations scattered over the range of the local race. Some stations were less than a city block apart; others were three or four miles apart. At least two families were grown from each station and were established in the nursery, one family to a row, in six adjacent blocks of ten rows each.

2. A pair of families arbitrarily referred to as race No. 6 and race No. 9 were originally considered to be collections of different races but proved to be similar. The two collections were made from wild plants about a half mile apart in Contra Costa County, just north of Berkeley. A detailed comparison was made between the two families of 75 plants each in order to determine whether or not they belonged to the same race. The families were planted in the summer in large flats in the greenhouse and bloomed from late fall to early spring. In addition, crosses were artificially made involving plants of No. 6 and No. 9 with a third race, No. 8, which was strikingly different.

3. The Field A collection consisted of a group of 70 families all taken from a single field in the Berkeley Hills in which five races were growing in close proximity. The purpose of this collection was to detect interracial crossing under natural conditions. The races were designated A-1, A-2, A-3, A-4, and A-5, three of which exhibited minor variations which were sufficiently stable for the detection of segregation. Race A-1, types one, two, and three, had the same general aspect and growth habits, but differed in color and hairiness of leaves, while A-2 and A-3 had hairy and smooth-leaved variants (table 3).

4. The Arizona collection was composed of a

TABLE 1. Quantitative comparison of strain No. 6 and strain No. 9 based on a family of 15 plants each.

Characters	Units	No. 6	No. 9
Seedling characters			
Days from germination to emergence of 2nd leaf.....	No.	15.6 ± 0.3	16.6 ± 0.3
Days between emergence of 2nd and 3rd leaves.....	No.	8.5 ± 0.1	8.5 ± 0.1
Days between emergence of 3rd leaf and 1st branch.....	No.	11.8 ± 0.39	11.8 ± 0.32
Length of 1st leaf at emergence of 2nd leaf.....	cm.	8.41 ± 0.19	7.86 ± 0.24
Length of 1st leaf at emergence of 3rd leaf.....	cm.	9.21 ± 0.21	8.36 ± 0.26 ^a
Width of 1st leaf at emergence of 2nd leaf.....	mm.	1.26 ± 0.04	1.03 ± 0.02 ^a
Length of coleoptile at emergence of 2nd leaf.....	cm.	1.38 ± 0.03	1.45 ± 0.04
No. of veins in 1st leaf at emergence of 2nd leaf.....	No.	3.6 ± 0.08	3.1 ± 0.03 ^a
Length of 2nd leaf at emergence of 3rd leaf.....	cm.	14.30 ± 0.26	13.79 ± 0.33
Width of 2nd leaf at emergence of 3rd leaf.....	mm.	2.02 ± 0.08	1.84 ± 0.04
Twisting of 2nd leaf measured in ¼ turns.....	No.	4.5 ± 0.3	5.0 ± 0.3
Height at emergence of 1st branch.....	cm.	20.91 ± 0.34	22.09 ± 0.53
Number of leaves at emergence of 1st branch.....	No.	4.1 ± 0.07	4.0 ± 0.05
Location of 1st branch (No. of node).....	No.	1.4 ± 0.08	1.5 ± 0.06
No. of tallest leaf (sequence designation).....	No.	2.09 ± 0.04	2.9 ± 0.04
Diameter of stem at emergence of 1st branch.....	mm.	1.42 ± 0.02	1.46 ± 0.02
Adult characters			
Number of branches at 52 days.....	No.	2.6 ± 0.1	3.4 ± 0.1 ^a
Number of branches at 70 days.....	No.	4.8 ± 0.2	14.9 ± 0.5 ^b
Number of branches at harvest.....	No.	10.8 ± 0.6	17.4 ± 0.7 ^b
Length of culm at harvest.....	cm.	85.7 ± 1.7	83.1 ± 1.6
Length of largest panicle on each plant.....	cm.	18.9 ± 0.4	16.9 ± 0.4 ^a
Length of uppermost internode of culm.....	cm.	46.8 ± 1.3	44.8 ± 0.6
Length of spikelets.....	cm.	2.74 ± 0.03	2.41 ± 0.02 ^a
Number of flowers per spikelet.....	No.	5.2 ± 0.1	4.0 ± 0.1 ^a
Number of nodes per panicle.....	No.	6.6 ± 0.1	6.8 ± 0.1
Number of nodes per culm.....	No.	4.6 ± 0.1	4.6 ± 0.1
Height of leaves at harvest.....	cm.	65.8 ± 0.8	63.9 ± 0.7
Width of panicle.....	cm.	13.2 ± 0.5	13.4 ± 0.5
Length of lemmas.....	mm.	16.0 ± 0.2	16.4 ± 0.2
Length of awns.....	mm.	8.6 ± 0.1	8.7 ± 0.1
Number of plants flowering at 10 weeks.....	No.	75	14 ^b

^a Difference significant.^b Difference highly significant.

series of 100 families from 22 stations in central and northern Arizona. One to 13 families were grown from each station in an attempt to detect segregation and interracial hybridization. More than one race could be distinguished when making collections at a number of stations and a special effort was made to get representatives of the different races. Each station was represented by its own local race or races except stations 101, 102, and 103 which were but a few miles apart along the Gila River near Sacaton. The Arizona collection was found to include two distinct species, a duodecaploid *B. arizonicus* (Shear) Stebbins (Stebbins, Tobgy and Harlan, 1944) and an octoploid related to *B. marginatus* Nees, both species occurring at several of the stations.

In addition certain families of well-known and familiar races were grown in the nursery as checks.

RESULTS.—*Race No. 2.*—Plants within a family proved to be extremely uniform. The families from a single station or from stations a short distance apart were also very similar, with two exceptions. Families from stations some distance apart were more dissimilar. The entire collection, however,

maintained a marked individuality. All the families undoubtedly belonged to race No. 2 and maintained those vegetative features which distinguished that race from other local races.

The stations were numbered 201 to 219. Descriptions of a few of them may further explain the above-mentioned generalizations. Two families that were grown from station 201 located at California Street and University Avenue in Berkeley appeared to be identical in every respect. Station 202 was located two blocks to the north of California Street. No differences were discovered between the two families of station 202 and they could not be distinguished from those of station 201. Station 203 was located nearly a mile to the north in a vacant lot. The three families grown from this station appeared to be identical with each other, but were shorter than those of the previous stations and flowered nearly three weeks later. Station 204 was located about a block away from station 203 and the families of that station resembled those of station 203 in height and date of flowering.

Stations 206, 207, and 208 were located at different sides of a single city block. Three families were

grown from each of these stations. The families from 206 and 207 were apparently identical, but station 208 had two distinct family types. Two of the families from station 208 flowered three weeks earlier than the other family, which flowered at the same time as the families from stations 206 and 207. Other stations exhibited similar results. As many as ten families were grown from one station without showing any consistent interfamilial differences, while interstation differences were frequently detected. Two stations had representatives of two types of families. Plants within a family were always strikingly uniform.

It may be concluded on the basis of this simple experiment that a given recognizable local race is not completely uniform genetically. None of the families in the No. 2 collection would be mistaken for any other race. The general uniformity of the entire collection is striking when contrasted with other races, but small differences between families from different stations and sometimes between families from the same station were found. These variations consisted of small differences in height of the leaves, time of flowering, and subtle quantitative differences in the size of panicle, size of spikelet, and other characters. The aspect of the plants remained unmistakably that of race No. 2, the diagnostic characters of white, hairy sheaths; spikelet color pattern; and stiff, erect leaves being common to all plants in the collection.

Races No. 6 and 9.—The results of measurements on 31 quantitative characters are shown in table 1. Many of these "characters" are actually different measurements of the same general character. The first three, for instance, in which No. 6 differs significantly from No. 9, refer to the relative size of the first leaf. Number 6 apparently has somewhat longer and broader first leaves and more veins because of the greater leaf width. The next group of three characters which differ significantly are also measurements of the same aspect of growth habit, i.e., the branching character. Number 9 branches sooner and more profusely than does No. 6. The length of the spikelets and the number of flowers per spikelet are also related. Number 6 apparently has somewhat larger spikelets than No. 9. It also has a slightly larger panicle. Therefore, No. 6 shows measurable differences from No. 9 in respect to five characters, as follows: it has larger first leaves, larger spikelets, larger panicles, fewer branches and more regular flowering than No. 9.

The differences mentioned are largely such as are readily susceptible to environmental modification. Although attempts were made to keep the environment as uniform as possible, it is, of course, impossible to maintain identical environments for two groups of plants under any circumstances. Soil was mixed carefully before being placed in the flats and care was used in watering and transplanting, but conditions in the two flats were undoubtedly somewhat different. How much of the difference in growth can be ascribed to the effect of environment

is impossible to state. Plants of No. 6 and No. 9 were grown together repeatedly during the course of this and other experiments, the impression being that the leaves of No. 9 were usually somewhat more slender than those of No. 6. Although this was not effectively demonstrated by the measurements, it is believed that the significant differences obtained in this respect are genetical.

The difference in branching undoubtedly represents a highly significant genetical variation between the two numbers. No relatively small difference in environment is likely to produce so large a difference in number of branches. Other lots of seed of these numbers showed the same results, i.e., No. 9 branched sooner and more profusely than No. 6. The differences in panicle and spikelet size were perhaps less significant. It would be difficult indeed to separate a mixture of No. 6 and No. 9 on the basis of these characters. In fact, the two numbers looked so much alike at maturity that no satisfactory method of telling them apart was found. Even the difference in branching is not so striking at maturity, and being a variable character, branch numbers of the two types overlap. To say with certainty that a particular plant was No. 6 or No. 9 without knowing its origin would be difficult in extreme cases and impossible in many others.

The difference in flowering habit is real and genetical (table 2). Number 6 flowered promptly in six to seven weeks after planting so that by ten weeks after planting all 75 plants had flowered. Number 9 began to flower at about the same time as No. 6, but with marked reluctance. By ten weeks after planting, only 14 of the plants had flowered. When No. 6 and No. 9 were crossed with No. 8, the early flowering habit appeared to be dominant. The No. 8 \times No. 6 F_1 plants flowered with the No. 6 controls or very slightly afterwards, while the No. 8 \times No. 9 F_1 plants showed marked reluctance to flower during the winter. A few of these hybrids produced one or two very poor panicles, but most of them remained in vegetative state until spring. The No. 8 \times No. 9 plants bloomed before the No. 8 controls, but much less profusely than did the No. 8 \times No. 6 plants, showing a real genetic difference in flowering habit between No. 6 and No. 9.

TABLE 2. Time from germination in August to full flowering.

Race No.	First emergence	Full flowering	No. of plants
No. 6	6 weeks	10 weeks	35
No. 8	25 weeks	27 weeks	35
No. 9	6 weeks	20 weeks	35
No. 8 \times No. 6....	7 weeks	12 weeks	18
No. 8 \times No. 9....	8 weeks	26 weeks	24

The two families did not, however, exhibit any greater differences than those between families of race number 2. They resemble each other more than they resemble families of any other race grown for

TABLE 3. *Uniform and segregating families taken from field A.*

Race	Type	Most distinctive characters	Number families		
			Uniform	Segregating	Total
A-1	1	Medium height; leaves hairy, green; panicle erect..	12	7	19
A-1	2	Medium height; leaves glabrous, green; panicle nodding; rosette dense	5	3	8
A-1	3	Medium height; leaves glabrous, bluish; panicle nodding; rosette lax.....	2	1	3
A-2	1	Medium height; leaves hairy; panicle erect, green..	2	0	2
A-2	2	Medium height; leaves glabrous; panicle erect, green	3	1	4
A-3	1	Medium height; leaves hairy, panicle erect, reddish	3	3	6
A-3	2	Medium height; leaves glabrous; panicle erect, reddish	1	1	2
A-4	1	Tall, slender; panicle fine, spikelets small, early flowering	14	0	14
A-5	1	Tall, robust; panicle open, spikelets large.....	12	0	12
Total			54	16	70

observation. The families, therefore, are designated as belonging to race number 6-9 and constitute another example of intraracial variation.

Field A collection.—The results of the population analysis of the seventy families taken from field A are shown in table 3. Most of the families were uniform, even though all were obtained from seeds taken from open-flowered panicles, many of which flowered not more than a foot or two away from a plant of another race or type. The segregating families were placed arbitrarily into one class. They included some families which were completely uniform except for one or two off-type plants, and other families which showed complete segregation. It was not always possible to distinguish these families from partially segregating families and, therefore, all families indicating heterozygosity were lumped together. In cases where the family was uniform for one or two plants of a different type, the inference is that the parent was essentially homozygous and the off-types were the result of out-crossing in the panicle from which the family was obtained. Most of the interbreeding in race A-1 was between type 1 and type 2 of this race. One natural cross was found between A-2 and A-3. Races A-4 and A-5 were apparently uniform, and no evidence of interbreeding was detected.

Race A-4 is the most distinctive race in the field, being tall with a very fine panicle bearing tiny spikelets and seeds. A-4 is an early race and passes its peak flowering period before the other races reach theirs. This is perhaps the main reason why not a single segregating family was found nor were intermediate forms observed in the field. Isolation in this case may be caused by differences in time of flowering.

Sixteen families exhibited segregation or the presence of off types. It must be emphasized however, that a special effort was made in collecting to get the intermediate types. The 70 families were not collected at random and, therefore, gave no indication as to the actual amount of natural interbreeding. It must also be recalled that the panicles from

which the families were derived were all chasmogamous panicles which permit cross pollination. Furthermore, the plants are perennials and intermediate forms could have been established over a period of years so that the amount of interbreeding in any one season must be considered substantially less than that indicated by the experiment.

It must be concluded, therefore, that despite the close proximity of chasmogamous panicles of different races in field A, and despite the several intermediate forms found in that field, the actual amount of interbreeding in any one season must be rather low. The fact that the discontinuities are preserved and that there is no continuous variation in the population in the field would indicate that free interbreeding does not exist.

The Arizona collection.—The extent of interracial hybridization detected in this sample of 100 families is indicated in table 4. To test further the uniformity and homozygosity of several of these families, two or more of the most divergent plants were selected from some of these families and small subfamilies grown from them. Comparisons of these subfamilies gave some indication as to the amount of heterozygosity of the original plants and indicated whether the variation found in a family was genetical or environmental.

The following subfamilies of 15 plants each were grown in the greenhouse.

101-2, two subfamilies	123-8, two subfamilies
101-3, three subfamilies	125-4, two subfamilies
102-2, two subfamilies	126, two subfamilies
103-2, two subfamilies	129-1, two subfamilies
106-1, two subfamilies	131-2, four subfamilies
111-2, two subfamilies	131-4, two subfamilies
123-2, two subfamilies	

Plants in all sets of subfamilies were strikingly uniform within subfamilies and between subfamilies of a given station, with the exception of one of the families taken from a 131-2 plant. This subfamily deviated somewhat in appearance from the other three families in the set of four and was in itself

TABLE 4. *Uniform and segregating families in the Arizona collection.*

Station	Uniform	Segregating	Total
101	5	0	5
102	3	0	3
103	1	0	1
104	2	0	2
105	3	0	3
106	2	1	3
107	1	0	1
108	5	0	5
109	1	0	1
110	1	0	1
111	3	2	5
112	11	2	13
113	9	0	9
115	4	0	4
116	4	0	4
123	6	3	9
124	7	0	7
125	8	0	8
126	1	0	1
129	3	1	4
130	2	2	4
131	5	2	7
Total	87	13	100

irregular, clearly indicating heterozygosity of the parent plant for a number of characters.

In this collection, as in the collection from field A, two types of segregating families were distinguished. Some families appeared to be completely uniform except for one or two plants which were strikingly different from the rest. Other families showed complete segregation and a great amount of variation. In a few cases the two types of segregating families could not be clearly distinguished and therefore the two classes were grouped together.

Among the families of the Arizona collection several well-known races were planted as checks. These were local Berkeley races numbers 2, 5, and 6. The nursery was established in the summer of 1941 and plants of No. 6 flowered that fall together with some of the Arizona races. Plants of No. 5 and No. 2 and the remainder of the Arizona collection did not flower until the following spring. In one of the No. 6 families, one plant stood out strikingly from the rest because of its height and erect habit. Number 6 was naturally subprostrate while the off-type plant was perfectly erect.

Since the history of this family of No. 6 was known, it was suspected at once that the off-type plant was a hybrid between races No. 6 and No. 5. The hybrid suspect was intermediate in appearance between these two races except that it had the erect habit of race No. 5. As a final check, a No. 6 \times No. 5 hybrid and its reciprocal were obtained artificially and comparisons made in F_1 and F_2 . The off-type plant occurring in the No. 6 family was proved in this manner to be a natural hybrid between No. 6 and No. 5.

Six other families of No. 6 were grown from plants which had equal opportunities for crossing with No. 5, but without detecting another hybrid. That natural interracial hybrids do occur has been effectively demonstrated, but that they occur infrequently is indicated by the evidence of these No. 6 families and by the infrequent detection of segregation in the Arizona and the field A collections.

DISCUSSION.—On the basis of the population analyses reported above, two unusual features of the variation pattern in the *Bromus carinatus* complex stand out clearly. The first is the extreme uniformity of most families taken from natural stands. Comparing the uniformity of these families with families taken from wild plants of other species and genera of grasses, it is concluded that the homozygosity of individuals belonging to different races of *B. carinatus* is of an unusual order among grass species. The second feature is the existence of swarms of races as found at Berkeley. In most grass species hundreds of races are not found in such a limited area. These features are probably interrelated and may be explained by the following hypothesis of the breeding structure.

The reproductive functions of the plant are carried on for the most part by self-pollinated flowers either from cleistogamous or chasmogamous panicles. The resultant inbreeding results in a rapid approach to complete homozygosity, and in strict limitation of the genetic variability of a race. Interracial hybrids occur occasionally through the chasmogamous panicles. The result is a highly heterozygous F_1 . Through selfing, this heterozygosity is rapidly reduced and in a few generations one to several new recombinations of the original characters become established as new, uniform races or types restricted in genetic variability. Many of the recombinations which are potential races or types will not become established, but there is a good chance that a cluster of types will be established, differing in only a few characters. Such a cluster would constitute a race such as race No. 2 or race No. 6–9. Thus, a swarm of variants and clear discontinuities between them may be obtained.

The relationship of this breeding structure based upon the habit of facultative cleistogamy to certain other recognized breeding structures might be pointed out. Two opposing forces are at work in the facultatively cleistogamous breeding structure: (1) intense inbreeding resulting from self-fertilization and (2) cross-fertilization. The variation pattern was intermediate between the patterns of variation exhibited by exclusively self-fertilized species and those which are exclusively cross-fertilized, and it exhibited some features of each.

The intense inbreeding resulting from self-pollination produces much the same kind of racial swarm as apomixis. In certain apomictic groups the natural forces which normally operate to form species do not function. Instead, a swarm of minute, apomictic races is formed which constitutes a complex. In their monograph on the American species of *Crepis*,

Babcock and Stebbins (1938) found that such complexes are not subject to the normal taxonomic treatment since true species are not formed. The whole group is better left as an apomictic complex. The complex described in this work bears many resemblances to an apomictic complex, but two fundamental differences must be noted. First, the individuals of the apomictic complex are highly heterozygous. Second, the apomictic races are closed units, not subject to genetical modification except by mutation, while the cleistogamous races are not closed units and can occasionally cross with one another. (See Dobzhansky, 1941, p. 378.)

Another pattern of variation which shows some similarities to that found in *B. carinatus* has been reported for species of *Poa* which are facultatively apomictic (Müntzing, 1940). Families of this genus taken from nature show striking uniformity, such as that demonstrated in families of *B. carinatus*. Occasionally a family will produce a strikingly different plant as a result of true fertilization. This pattern of variation is similar to that reported above in *B. carinatus* but the genetical cause is fundamentally different. The individual plants of *Poa* are highly heterozygous but the families appear uniform due to apomixis, and the off-type plants are due to a sexual combination of different gametes from the heterozygous parent. The individual plants of *B. carinatus* are highly homozygous and the families appear uniform for that reason, while the off-type plants are the result of sexual combination of unlike gametes from two different homozygous parents. Apomictic forms such as *Poa* spp. described above often have pronounced meiotic disturbances, while the self-pollinated are essentially regular at meiosis.

Other species which exhibit facultative cleistogamy do not necessarily form a complex of the kind described in this paper. In the case of certain *Stipa* spp. the habit of facultative cleistogamy is similar to that reported in *Bromus*, but self-pollination appears to be much less important in the reproductive functions of the plant. Families taken from nature show considerable variability, indicative of cross-pollination, and racial swarms similar to those of *Bromus carinatus* are apparently not formed.

The taxonomic aspect of the type of breeding structure reported here can be summed up in the words of Dobzhansky (1941), pp. 378, 379: "It is not surprising that the groups of organisms recognized as being uncommonly 'difficult' from the standpoint of delimiting species have proved to be mainly those in which asexual reproduction or self-fertilization are the only, or the predominant, modes of propagation. . . the species as a category which is more fixed and therefore less arbitrary than the rest is lacking in asexual and obligatorily self-fertilized organisms. All the criteria of species distinction utterly break down in such forms."

It would seem, therefore, that as long as the habit of facultative cleistogamy persists with self-pollinated flowers predominating in reproduction, clearly

delimited species within the complex will not be found. Moreover, the breeding structure as reported in the *Bromus carinatus* complex is not the only cause of variation. Four different chromosome numbers have been reported with the strong possibility of allopolyploidy involving some form outside of the *Ceratochloa* section. Stebbins and Tobgy (1944) have also reported that races from different localities, even when they have the same chromosome number, are likely to produce partly or wholly sterile hybrids, while slight reduction in fertility may be found in certain hybrids between races of the same locality. Much more work must be done before the whole geographic pattern of variation can be unraveled. It is considered that the present study of the local breeding structure is a desirable preliminary to the investigation of the whole problem in this genus.

SUMMARY

A total of 232 families taken from as many individual wild plants were analyzed for evidence of variation within a local race and for interbreeding between races.

Plants within a family were generally extremely uniform, indicating a high degree of homozygosity for individual wild plants.

Differences exhibited between families of the same local race indicate that a given race is not genotypically uniform.

Some families clearly showed segregation indicative of interracial hybridization.

An hypothesis for the natural breeding structure in *Bromus carinatus* is presented involving the habit of facultative cleistogamy and explaining the existence of swarms of local races. Self-pollination is the rule, but highly heterozygous interracial hybrids occur occasionally in chasmogamous panicles. Through selfing, this heterozygosity is rapidly reduced, and in a few generations one to several new recombinations of the original characters become established as new, uniform races restricted in genetic variability.

SOUTHERN GREAT PLAINS FIELD STATION,
WOODWARD, OKLAHOMA

LITERATURE CITED

- BABCOCK, E. B., AND G. L. STEBBINS, JR. 1938. The American species of *Crepis*. Carnegie Inst. Washington Publ. No. 504. 199 pp.
- DOBZHANSKY, T. 1941. Genetics and the origin of species. New York, Columbia Univ. Press. 2nd Ed. 446 pp.
- HARLAN, JACK R. 1945. Cleistogamy and chasmogamy in *Bromus carinatus* Hook. and Arn. Amer. Jour. Bot. 32: 66-72.
- HITCHCOCK, A. S. 1935. Manual of the grasses of the United States. U. S. Dept. Agric. Misc. Publ. No. 200. Washington, D. C.
- MÜNTZING, A. 1940. Further studies on apomixis and sexuality in *Poa*. Hereditas 26: 115-190.
- SHEAR, C. L. 1900. Studies on American grasses. A revision of the North American species of *Bromus*

occurring north of Mexico. U. S. Dept. Agric. Div. Agrost. Bull. 23: 1-66.
 STEBBINS, G. L., JR., AND H. A. TOBGY. 1944. The cytogenetics of hybrids in *Bromus*. I. Hybrids within

the section *Ceratochloa*. Amer. Jour. Bot. 31:1-11.
 ———, ———, AND J. R. HARLAN. 1944. The cytogenetics of hybrids in *Bromus* II. *Bromus carinatus* and *Bromus arizonicus*. Proc. Calif. Acad. Sci. 24:307-322.

THE STRUCTURE OF THE CELL WALLS OF *ASPERGILLUS* AND THE THEORY OF CELLULOSE PARTICLES¹

Edward S. Castle

IN RECENT years a comprehensive theory of the formation of the plant cell wall has been proposed by Farr (review: Farr, 1944), important features of which are as follows: the cellulose framework of the wall is composed of microscopic particles of crystalline cellulose of ellipsoidal shape and dimensions $1.5 \times 1.1 \mu$; the particles are formed in special plastids or in the cytoplasm where they are microscopically demonstrable singly or in short chains; growth of the wall occurs by transfer of these visible cellulose particles from the cytoplasm to the cell wall and their oriented deposition there.

The theory is based fundamentally on microscopic observations and microchemical tests. Visible cytoplasmic particles believed to contain cellulose were first seen by Farr in the conidiophores of *Aspergillus* (cf. discussion in Clark, 1934). Cellulose particles have also been described in a variety of other plant cells including the cotton hair, although their existence in cotton has not been confirmed by Anderson and Kerr (1938) or by Hock and Harris (1940). Because of the far-reaching implications of this theory and its bearing on cell wall studies in the fungi, the present paper repeats and extends the original observations of Farr and Eckerson (1934) on *Aspergillus*.

Cultures of *A. niger* and *A. clavatus*, kindly furnished by Dr. D. H. Linder, were used throughout the present study, maintained on the modified Czapek agar recommended by Thom and Church (1926). Observations were made both in ordinary and in polarized light of conidiophores at various stages of development, and most of the microchemical tests were carried out on slides under the microscope.

The young conidiophore of *Aspergillus* is an elongated cylindrical cell without cross walls, tapering to a blunt tip at the free, growing end. At this growing tip there is present only the primary wall, which does not exceed 0.5μ in thickness; below the tip the wall ceases to grow in area and is thickened by deposition of a secondary wall on its inner surface. The mature wall of the conidiophore may reach a thickness of 1.5μ , as found by Frey (1927). Farr and Eckerson observed that the wall of the young conidiophore was optically isotropic, while the older parts of the wall were anisotropic. From this fact and the ability of the young wall to take up ruthenium red they concluded that the young wall contained no cellulose and was composed of "pectic

substance," this later becoming lined with cellulose.

In the present study it was found that the wall has a resistant framework which remains intact after treatment designed to remove or destroy pectic substances. Even the delicate wall of the growing tip retains its integrity after treatment with dilute or concentrated alkali, or after heating in glycerine or saturated KOH. Furthermore, the young wall can be made feebly anisotropic by warming in dilute alkali to extract substances which mask its birefringence, and this birefringence can be enhanced by staining with iodine or Congo red. It is clear that the young wall, even at the growing tip of the cell, possesses a coherent structural skeleton which, unlike pectic substances, is highly resistant to alkali and shows evidence of oriented structure.

Farr and Eckerson appear to have taken the increasing birefringence of the thickened, older wall as evidence for the deposition of cellulose. There is, however, abundant evidence that the polysaccharide framework of the wall of *Aspergillus* is composed of chitin and does not contain cellulose (microchemical tests: van Wisselingh, 1925; Hopkins, 1929; Bucherer *et al.*, 1940; chemical analyses: Behr, 1930; Norman *et al.*, 1932; X-ray study: Khouvine, 1932; degradation by chitin-destroying bacteria: Bucherer, 1935). This distinction has been confirmed in the present study by solubility and microchemical tests. The untreated wall or the wall which has been pretreated with alkali remains intact, unswollen, and undissolved in cuprammonium solutions that dissolve cotton fibers in a few seconds. Walls pretreated with alkali stain readily with iodine, and addition of 60 to 70 per cent H_2SO_4 results in swelling but no trace of the blue coloration characteristic of cellulose. Walls heated to $160^\circ C$. in saturated KOH and treated with iodine and dilute H_2SO_4 according to the method of van Wisselingh (1925) take on the intense red-violet coloration characteristic of chitosan, a derivative of chitin formed by the action of hot concentrated alkali (Zechmeister and Tóth, 1939). Walls thus pretreated in saturated KOH dissolve almost instantly in one per cent acetic acid, a further property of chitosan (Campbell, 1929). The absence of any test for cellulose and the occurrence of generally accepted tests for chitosan support the prevalent view that the walls of *Aspergillus* contain chitin and do not contain cellulose. Even at the growing tip of the cell, these same tests show that the coherent, alkali-

¹ Received for publication November 27, 1944.

resistant skeleton of the wall is composed of chitin and not of cellulose.

It is generally believed that the structural scaffolding of most plant cell walls is largely composed of microfibrils of cellulose, strongly linked together and frequently overlying one another in various planes of preferred orientation (Bailey, 1940). Cell walls of the higher fungi commonly possess chitin instead of cellulose, although the mutual exclusiveness of these two substances has been questioned in certain cases (Nabel, 1939; Thomas, 1942). In some cellulose walls, as for example the cotton fiber, the microscopic structure is relatively coarse and individual microfibrils are clearly distinguishable; in others, including the cell walls of fungi where chitin is present, microfibrils cannot easily be resolved.

Farr and Eckerson described and illustrated in the thickened walls of *Aspergillus* conspicuous fibrils arranged in spirals and showing occasional changes in orientation ("reversals") exactly as do the secondary wall fibrils in the cotton hair. The writer has searched without success for evidence of such visible, spirally oriented fibrils and for reversals of fibrillar orientation. Between crossed nicols the thickened walls are bright but, unlike those of cotton, they extinguish sharply and completely. Among the figures of *Aspergillus* published by Farr and Eckerson only two show reversals and visible fibrils arranged in spirals, and these two are the only figures which do not include vesicles, foot-cells, or other characteristic structures confirming their fungous origin. The writer is driven to the conclusion that these two figures which show crossed spirals and reversals typical of cotton are not of *Aspergillus* but of cotton lint which often contaminates microscopic preparations. No such structures are visible in any conidiophore of *Aspergillus* studied by the writer, or verifiable as *Aspergillus* in the published figures of Farr and Eckerson. Unlike cotton, the texture of the wall structure in *Aspergillus* is comparable to that found in the sporangio-phore of *Phycomyces* (Castle, 1938b), where varied lines of evidence also show that the wall framework is composed of chitin (von Wettstein, 1921; Diehl *et al.*, 1935; van Itersen *et al.*, 1936; Schopfer, 1937).

If cellulose does not occur in the cell walls of *Aspergillus*, the demonstration of particles of crystalline cellulose in the cytoplasm would be of special interest. In *Aspergillus*, as in all plant cells, the cytoplasm often appears granular. Granules are particularly prominent toward the apical end of the conidiophore prior to spore formation. Farr and Eckerson found that cytoplasmic granules in the conidiophore could be stained by ruthenium red, and described these as cellulose particles each surrounded by a coating of pectic substance. The cellulosic nature of these particles was not shown by any chemical test, and appears to have been inferred from the supposed existence of cellulose in the cell wall. Although the cell contents readily take up iodine and become dark and granular, at no time in

the present study has it been possible to obtain a blue coloration of cytoplasmic granules by the use of iodine and H_2SO_4 , even after the alkali treatment recommended by Farr and Eckerson for removal of pectic coatings. Nor were indications found by examination in polarized light of any visible anisotropic particles in the cytoplasm, or of linear chains of such particles.

DISCUSSION.—Although the theory of cell wall formation by cellulose particles appears to have grown from observations on *Aspergillus*, the consensus of opinion is that the walls of this fungus have a structural skeleton of chitin and do not contain cellulose. The chitin content of the walls has been estimated to be from a few per cent to as high as 20 per cent (Norman, 1937; Rippel, 1937), and has been found to vary with age and with the conditions of culture (Behr, 1930). The difficulty of completely extracting the cell contents makes such estimates uncertain, and the special difficulty of preparing pure chitin from plant sources suggests that other constituents are intimately admixed with chitin in the cell walls. Repeated extraction of *A. niger* with hot water and hot dilute alkali appears necessary to free its chitin from soluble substances (Behr, 1930) and to obtain a sharp X-ray diagram (Khouvine, 1932). The nature of these extractable substances is uncertain, although the alkali-soluble fraction includes Mangin's "callose" which, according to Norman (1937), is some form of glucose polysaccharide. Such substances cannot constitute the structural framework of the cell wall, since the wall hangs together tenaciously after their removal. In the resistant cell wall material of *A. fischeri*, Norman and Peterson (1932) found in addition to chitin a variable non-nitrogenous fraction, but as judged by several criteria there was no evidence of the presence of cellulose.

Farr and Eckerson were led by the apparent optical isotropy of the young wall to conclude that no polysaccharide framework was present and that at this early stage the wall was composed of "pectic substance." The present microchemical tests show that even the young wall of the conidiophore has a structural skeleton of chitin, and examination in polarized light shows that this skeleton is weakly but definitely anisotropic when substances which cancel or conceal its birefringence are extracted from the wall. For students of morphogenesis there is, however, a further important objection to the view implied by Farr and Eckerson that the young wall is solely composed of isotropic pectic substances. If this were the case, there could be no possible basis for the cylindrical form and growth of the cell. Nägeli pointed out many years ago that in growing cells which are not subject to external mechanical influences, continuing deviation from the spherical form implies the existence of directional properties in the wall (*cf.* Thompson, 1942). Such properties are manifested in the polarized growth of certain types of cells, which can be understood only in terms of oriented structure in the growing wall.

Optical evidence of such oriented structure in growing, primary walls has been assembled by Frey-Wyssling (1935, 1936), who calls attention to the distinctive optical character and anisotropy of the primary wall of tubular cells. This evidence is interpreted to mean that the average orientation of micelles or microfibrils in the primary wall is more nearly transverse than parallel to the long axis of the cell. Such an interpretation has been amply confirmed by subsequent studies of the orientation of visible systems of microfibrils in the primary wall of cotton (Anderson *et al.*, 1938; Hock *et al.*, 1941), and by polarized light studies of the hairs of the stamens of *Tradescantia* (van Iterson, 1937) and of the sporangiophore of *Phycomyces* (Castle, 1938b). A possible mode of origin of such oriented structure in primary walls has been discussed (Castle, 1937b; van Iterson, 1937; Diehl *et al.*, 1939). Perhaps the clearest demonstration that the primary walls of cells not encompassed in a tissue possess unmistakable directional properties is given by studies of the oriented growth of the sporangiophore of *Phycomyces* (Oort, 1931; Castle, 1936a, 1936b, 1937a, 1938a, 1940, 1942). Here, quite apart from *a priori* considerations, we have direct physiological and morphological evidence that precludes any view of the basic primary wall structure as optically or otherwise isotropic.

In assuming that the marked birefringence of the older wall necessarily indicated the deposition of cellulose, Farr and Eckerson were in error, since the birefringence of plant and animal structures composed of chitin is well known (Schmidt, 1939). The writer has been unable to confirm the presence

in the secondary wall of *Aspergillus* of conspicuous spiral fibrils such as occur in the cellulose wall of the cotton fiber. As reported by Frey (1927), the mature wall of the conidiophore shows strong birefringence which is positive with respect to the long axis of the cell, and sharp parallel extinction.

Frey estimated the thickness of the hyphal walls to be from 0.2 to 0.3 μ ; the entire wall at the growing tip of the conidiophore is not over 0.5 μ in thickness. Clearly such walls cannot be built of anisotropic particles, the minimum dimension of which is greater than 1 μ . Bailey (1940) has pointed out that many cell walls containing cellulose are much less than one micron in thickness, with individual lamellae as thin as 500 Å. Such facts are irreconcilable with the theory under discussion.

SUMMARY

Both the primary wall at the growing tip of the conidiophore of *Aspergillus* and the older, thicker secondary wall have a coherent, fine-textured structural skeleton of chitin. There is no tenable evidence that the walls contain cellulose, or that identifiable particles of cellulose or of chitin exist in the cytoplasm. At the growing tip the entire cell wall is less than 0.5 μ in thickness and cannot be constituted of particles 1 μ or more in diameter. Theories of cell wall formation by the deposition of visible cytoplasmic particles of cellulose are not supported by these facts.

BIOLOGICAL LABORATORIES,
HARVARD UNIVERSITY,
CAMBRIDGE, MASSACHUSETTS

LITERATURE CITED

- ANDERSON, D. B., AND T. KERR. 1938. Growth and structure of cotton fiber. *Indust. and Engin. Chem.* 30: 48-54.
- BAILEY, I. W. 1940. The walls of plant cells, in *The Cell and Protoplasm* (Pub. No. 14, Amer. Assoc. Adv. Sci.). The Science Press, Lancaster.
- BEHR, G. 1930. Über Autolyse bei *Aspergillus niger*. *Archiv f. Mikrobiol.* 1: 418-444.
- BUCHERER, H. 1935. Über den mikrobiellen Chitinabbau. *Zentralbl. f. Bakteriol. Parasitenkunde u. Infektionskrank., Abt. 2*, 93: 12-24.
- , AND W. SCHMIDT-LANGE. 1940. Chemische Untersuchungen am Tuberkellbazillus. *Archiv f. Hygiene u. Bakteriol.* 124: 298-303. (Seen in abstract only.)
- CAMPBELL, F. L. 1929. The detection and estimation of insect chitin, and the irrelation of "chitinization" to hardness and pigmentation of the cuticula of the American cockroach, *Periplaneta americana* L. *Annals Entomol. Soc. Amer.* 22: 401-426.
- CASTLE, E. S. 1936a. The influence of certain external factors on the spiral growth of single plant cells in relation to protoplasmic streaming. *Jour. Cell. and Comp. Physiol.* 7: 445-454.
- . 1936b. The origin of spiral growth in *Phycomyces*. *Jour. Cell. and Comp. Physiol.* 8: 493-502.
- . 1937a. The distribution of velocities of elongation and of twist in the growth zone of *Phycomyces* in relation to spiral growth. *Jour. Cell. and Comp. Physiol.* 9: 477-489.
- . 1937b. Membrane tension and orientation of structure in the plant cell wall. *Jour. Cell. and Comp. Physiol.* 10: 113-121.
- . 1938a. The effect of torque on the axis of spiral growth in *Phycomyces*. *Jour. Cell. and Comp. Physiol.* 11: 345-358.
- . 1938b. Orientation of structure in the cell wall of *Phycomyces*. *Protoplasma* 31: 331-345.
- . 1940. Discontinuous growth of single plant cells measured at short intervals, and the theory of intussusception. *Jour. Cell. and Comp. Physiol.* 15: 285-298.
- . 1942. Spiral growth and reversal of spiraling in *Phycomyces*, and their bearing on primary wall structure. *Amer. Jour. Bot.* 29: 664-672.
- CLARK, G. L. 1934. The macromolecule and the micelle as structural units in biological materials with special reference to cellulose. Cold Spring Harbor Symp. Quant. Biol. 2: 28-38.
- DIEHL, J. M., AND G. VAN ITERSON. 1935. Die Doppelbrechung von Chitinsehn. *Kolloid Zeitschrift.* 73: 142-146.
- , C. J. GORTER, G. VAN ITERSON, AND A. KLEINHOONTE. 1939. The influence of growth hormone on hypocotyls of *Helianthus* and the structure of their cell walls. *Recueil trav. bot. Néerl.* 36: 709-798.
- FARR, W. K. 1944. Plant cell membranes, chapt. 29 in *Colloid Chemistry, Theoretical and Applied* (ed. J. Alexander), vol. 5. Reinhold, New York.

- , AND S. H. ECKERSON. 1934. Formation of cellulose membranes by microscopic particles of uniform size in linear arrangement. *Contrib. Boyce Thompson Inst.* 6: 189-203.
- FREY, A. 1927. La formation des cellules géantes du "*Sterigmatocystis nigra*." *Revue Gén. Bot.* 39: 277-305.
- FREY-WYSSLING, A. 1935. Die Stoffausscheidungen der höheren Pflanzen. Springer, Berlin.
- . 1936. Der Aufbau der pflanzlichen Zellwände. *Protoplasma* 25: 261-300.
- HOCK, C. W., AND M. HARRIS. 1940. Microscopic examination of cotton fibers in cuprammonium hydroxide solutions. *Jour. Research Nat. Bur. Stand.* 24: 743-748.
- , R. C. RAMSAY, AND M. HARRIS. 1941. Microscopic structure of the cotton fiber. *Jour. Research Nat. Bur. Stand.* 26: 93-104.
- HOPKINS, E. W. 1929. Microchemical tests on the cell walls of certain fungi. Cellulose and chitin. *Transact. Wisconsin Acad. Sci. Arts Letters* 24: 187-196.
- ITTERSON, G. VAN. 1937. A few observations on the hairs of the stamens of *Tradescantia virginica*. *Protoplasma* 27: 190-211.
- , K. H. MEYER, AND W. LOTMAR. 1936. Ueber den Feinbau des pflanzlichen Chitins. *Recueil trav. chim. Pays-Bas* 55: 61-63.
- KHOUVINE, Y. 1932. Étude aux rayons X de la chitine d'*Aspergillus niger*, de *Psalliota campestris* et d'*Armillaria mellea*. *Compt. Rend. Acad. Sci. Paris* 195: 396-397.
- NABEL, K. 1939. Über die Membran niederer Pilze, besonders von *Rhizidiomyces bivellatus* nov. spec. *Archiv f. Mikrobiol.* 10: 515-541.
- NORMAN, A. G. 1937. The biochemistry of cellulose, the polyuronides, lignin, etc. Oxford.
- , AND W. H. PETERSON. 1932. The chemistry of mould tissue. II. The resistant cell wall material. *Biochem. Jour.* 26: 1946-1953.
- OORT, A. J. P. 1931. The spiral-growth of *Phycomyces*. *Proc. Kon. Akad. Amsterdam* 34: 564-575.
- RIPPEL, A. 1937. Chitin bei Mikroorganismen. Eine Richtigestellung. *Biochem. Zeitschr.* 290: 444.
- SCHMIDT, W. J. 1939. Über den polarisationsoptischen Nachweis des Chitins bei Tieren und Pflanzen. *Zeitschr. f. Wiss. Mikroskop. u. f. mikroskop. Techn.* 56: 24-51.
- SCHOPFER, W. H. 1937. Recherches sur le métabolisme de l'azote d'un microorganisme acellulaire (*Phycomyces blakesleeanus* Bgf.). Le rôle des facteurs de croissance. *Protoplasma* 28: 381-434.
- THOM, C., AND M. B. CHURCH. 1926. The Aspergilli. Williams and Wilkins, Baltimore.
- THOMAS, R. C. 1942. Composition of fungus hyphae. III. The Pythiaceae. *Ohio Jour. Sci.* 42: 60-62.
- THOMPSON, D'ARCY W. 1942. On growth and form. Macmillan, New York.
- WETTSTEIN, F. VON. 1921. Das Vorkommen von Chitin und seine Verwertung als systematisch-phylogenetisches Merkmal im Pflanzenreich. *Sitzungsber. Akad. Wiss. Wien, math.-naturw. Kl., Abt. 1*, 130: 3-20.
- WISSELINGH, C. VAN. 1925. Die Zellmembran. Vol. III/2 of *Handbuch der Pflanzenanatomie*, Borntraeger, Berlin.
- ZECHMEISTER, L., AND G. TÓTH. 1939. Chitin und seine Spaltprodukte. *Fortschr. Chem. organ. Naturstoffe* 2: 212-247.

THE CYTOPLASMIC BASIS OF INTERCELLULAR PATTERNS IN VASCULAR DIFFERENTIATION¹

Edmund W. Sinnott and Robert Bloch

ONE OF the distinctive features of organisms is the possession of definite bodily forms or patterns, which are the result of controlled growth relationships during ontogeny. The external form of an organism or its parts is the outward expression of a series of inner developmental relationships between tissues of various types which produce the complex pattern of differentiated cells familiar to histologists. This pattern is the result of differences in the location and duration of cell division, in the planes where division occurs, in the amount and direction of cell expansion, in changes in the cell wall, and in other respects. What the factors are which produce these histological patterns is a major problem of morphogenesis.

The manner in which such patterns arise during development is often difficult to observe, since their important early stages occur in meristematic regions where the cells are small, delicate, and difficult of access. Much more favorable material is provided by tissues in which regeneration is taking place, for here patterns are reconstructed by cellular redifferentiation in regions where the cells are comparatively large and are mature or nearly so. The changes which

go on in such cells, destined at first to form one part of a tissue pattern but now altered in development to form quite another part, are relatively easy to observe. The regeneration, in presumptive fundamental tissue, of vascular strands and particularly of xylem cells with their distinctive wall markings, is an especially good example of this.

Such experimentally induced reconstitution of vascular bundles has repeatedly been described by Vöchting (1892), Freundlich (1909), Kaan Albest (1934), and especially Simon (1908). Among other experiments, Simon made transverse cuts in still immature internodes of *Coleus*, partially severing the vascular cylinder. Into these cuts he inserted mica plates. From the basal ends of the severed strands new bundles began to differentiate in the parenchyma of the pith; and, passing back of the incision, these ultimately joined the apical ends of the cut strands below, or joined intact lateral bundles. The origin, character, and course of these strands he found to differ depending on the age of the internode and the depth and position of the wound. Regeneration was generally basipetal. Freundlich (1909) studied the same phenomena around the severed veins of growing leaves, Kaan

¹ Received for publication December 4, 1944.

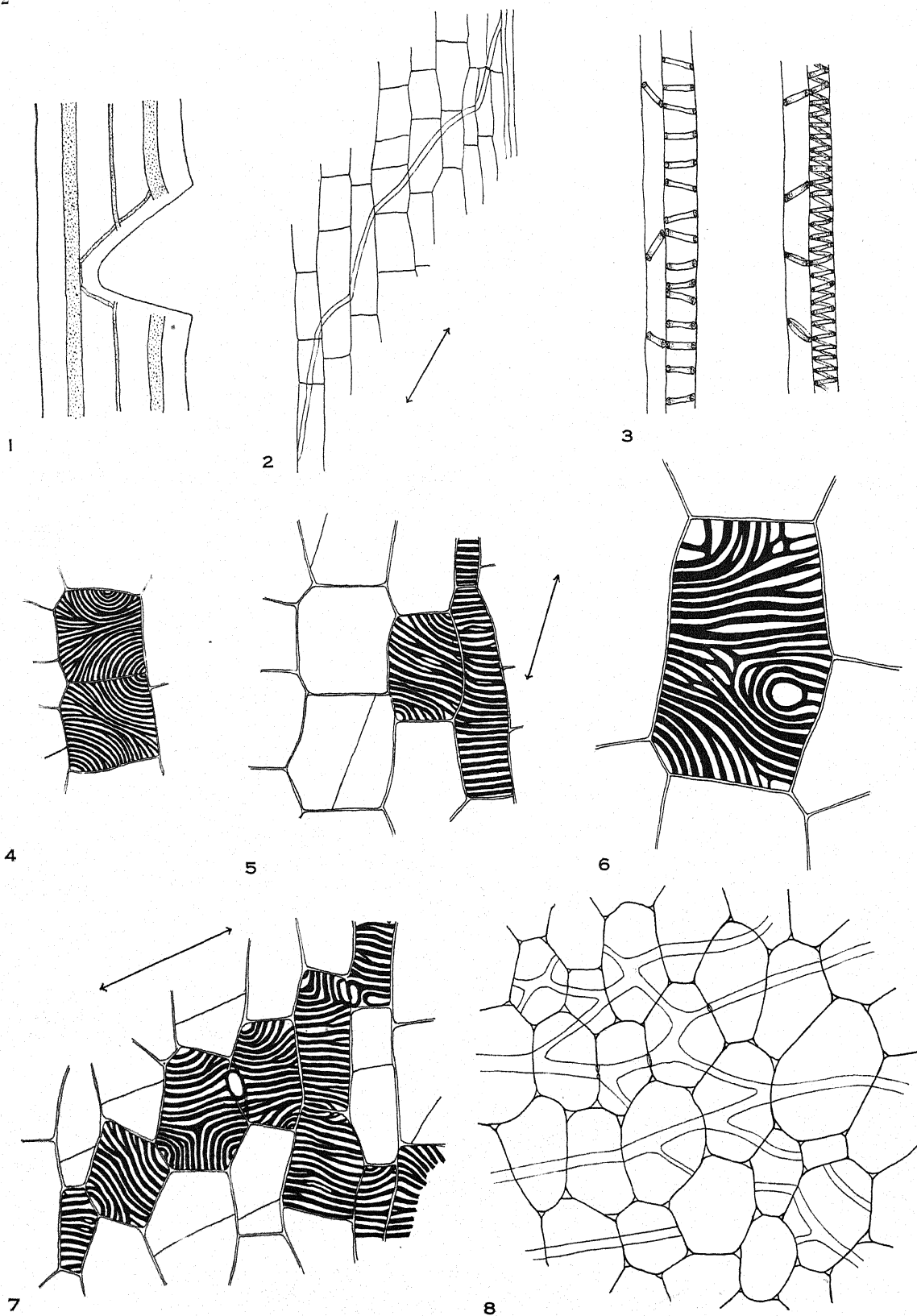


Fig. 1-8.—Fig. 1. Diagrammatic longitudinal section through internode of *Coleus* eight days after wounding, showing normal vascular strands (dotted) and oblique regenerated connection between them across the outer pith around

Albist (1934) in phloem strands in herbaceous stems, and both Vöchting (1892) and Simon (1908, 1930) in graft unions. These workers were concerned chiefly with the causes of strand regeneration but gave relatively little attention to the early steps in the process or to changes induced in the cells.

In such cases of bundle regeneration a description of the changes undergone by these cells of the fundamental tissues, when they become the seat of a secondarily induced vascular pattern, is necessary before any thorough analysis of the causes of these changes can be made. To furnish such a description, in some rather simple cases of the reconstitution of vascular strands in regeneration, is the purpose of the present paper.

METHODS.—Internodes of *Coleus hybridus* were investigated, beginning with the first which was freely visible (about two mm. long) and including the next three or four. The oldest studied had reached mature size but growth of their vascular cylinders was not yet complete.

The young internode is rectangular in cross section, each corner being occupied by a large bundle, with smaller strands of varying size on the four sides. Interfascicular cambium begins to form when the internode has reached its full length. The pith consists of large and thin-walled parenchyma cells, which were already vacuolate in the earliest stages studied.

Two oblique transverse cuts, varying in depth, were made in an internode, removing a wedge-shaped portion of the stem and interrupting more or less extensively its vascular system (fig. 1). Unlike Simon, who studied only the mature reconstructed strands after ten or fourteen days, the present writers examined the early developmental stages in strand formation and collected material daily from two to eight days after wounding. This was killed in CRAF, run through the butyl alcohol series, and stained with safranin and fast green. Series of sections were made transversely, radially, and tangentially.

RESULTS.—Reconstitution of xylem and phloem strands was often observed, and conformed to the description given by previous workers. The various

differences in strand formation, depending on internal and external factors and so adequately described by others, were not examined in detail. Particular attention was given to those cases where a corner bundle or one of the larger lateral bundles, when cut, reestablished connection between its two ends, or with other bundles, by the development of a vascular bridge in the pith. Other cases of bundle regeneration were also studied.

The first observable step in the formation of such a bridging strand is usually an increase in the density of cytoplasm in a series of cells along the course of the future bundle, as the cells prepare to divide. Division here is by the method of phragmosome formation previously described (Sinnott and Bloch, 1941a). The original axis of elongation of these pith cells is parallel to the axis of the internode; the plane of the new divisions, however, bears no relation to this axis but is parallel to the course of the future strand, which at this stage must therefore already be established.

In many strands these divisions are soon followed by others, with the result that a provascular strand is formed consisting of rather narrow cells elongated in the direction of the new bundle; and from these, xylem and phloem elements are differentiated as in normal development.

In the xylem, however, the daughter cells of the first division, still rather large, often fail to divide again but proceed to differentiate directly into tracheids. Less often, but by no means uncommonly, one or more of the cells in the course of the future strand fail to divide at all but, still preserving their original rectangular shape as pith cells, develop directly into xylem elements (fig. 7). Such cases were observed by Freundlich (1909) and others. Not infrequently a part of the xylem of a regenerating strand, sometimes extending for some distance, is composed of these metamorphosed pith cells, now ringed, spiral, or reticulate tracheids or vessels. They form a somewhat staggered row, but each is in contact with a similar element above and below or at either side.

It is these relatively large cells, transformed directly into xylem elements, which are of especial interest, because they are large enough so that the

the wound gap.—Fig. 2. Semi-diagrammatic drawing of a regenerated phloem strand in *Coleus* showing the row of phloem cells, whose course is independent of the original tissue axis, but continuous across a number of pith cells. $\times 125$.—Fig. 3. Protoxylem elements from *Zea mays*, showing ringed thickenings (left) and spiral thickenings (right). In both cases the lignified thickenings in one cell are directly opposite those in the adjacent one. Camera lucida drawing. $\times 100$.—Fig. 4. Portion of a regenerating strand showing two xylem cells differentiated directly from pith cells, the lignified bands forming a continuous pattern. Camera lucida drawing. $\times 300$.—Fig. 5. Portion of a regenerating xylem strand at edge of normal bundle (right), showing cell divisions parallel to the axis of the new strand (indicated by arrow) and lignified bands in pith cell arranged approximately at right angles to the course of the strand. Camera lucida drawing. $\times 300$.—Fig. 6. Single pith cell which has become a xylem cell, showing pattern of lignified bands and a pore. Camera lucida drawing. $\times 450$.—Fig. 7. Enlarged portion of a regenerating xylem strand as shown in figure 1, passing obliquely through pith parenchyma from edge of normal strand (right), showing pattern of lignified bands in walls and the position of pore areas. The new division walls and the line of pores are parallel to the course of the new strand (shown by arrow). Eight days after wounding. Camera lucida drawing. $\times 300$. The pattern of lignified bands on such cell walls forms a three-dimensional system, and its presentation in one plane will therefore vary to some degree according to the focal level.—Fig. 8. Transverse section through portion of cortex in air root of a *Cattleya* hybrid showing system of lignified bands (réseau de soutien) forming a continuous pattern. Camera lucida drawing. $\times 125$.

changes in them, associated with the differentiation of the new vascular strands, can most readily be seen. The most conspicuous of these changes is the development of lignified bands in rings or spirals; or, where lignification is more extensive, of pits and porous openings. The appearance of the bands is very different here from what it is in protoxylem or early metaxylem, where the cells are much elongated and pointed and the bands are all at right angles to the longer dimension of the cell. In these much wider cells the bands also tend to run at right angles to whichever dimension is the longer, but at the square ends of the cells this regular pattern is disturbed and the bands tend to cut across the corners in wide arcs, although the ones most distant from the ends of the cell may extend straight across it (fig. 6). Thus when a wall is seen in face view the bands usually meet the side walls (seen in section) at right angles. Each wall—transverse as well as longitudinal—therefore shares the characteristic pattern of thickenings and pits.

The bands in one cell are directly opposite those in the adjacent wall of the next cell, so that, as seen in longitudinal section, a complex pattern of curves is set up (fig. 4) passing across cell boundaries and somewhat resembling the "lines of force" in a magnetic field. It is noteworthy, however, that the general orientation of such a wall pattern is not a random one but conforms to the orientation of the axis of the new strand, for the lignified bands usually tend to be arranged at right angles to its course (fig. 5).

This relationship is especially evident, in cells which become vessels, in another feature of the characteristic lignified pattern, the pores. These when present are definitely related to the bands, each being, so to speak, an "eddy" in the band system (fig. 6). The pores are by no means confined to the transverse walls of these cells but may occur anywhere, and it is significant that they usually occupy such positions in the new strand that a line between the two pores of a vessel is parallel to the course of the new strand (fig. 7).

Thus in three respects—the plane of cell division, the orientation of the ringed and spiral markings, and the position of porous openings—there is evident the establishment of a new physiological axis in each cell which is to become part of the regenerated strand, an axis quite different from its original one and conforming to that of the strand which is being differentiated. Furthermore, the changes in each cell are definitely related, in a continuous pattern, to the changes in its neighbor cells.

This change in orientation is first evident in the cytoplasm of those cells which divide, since the cytoplasmic diaphragm, the earliest indication of the plane of division, is parallel to the axis of the strand. It is worthy of note that the other two indications of the new orientation (the distribution of thickenings in the wall and the location of pores) are also manifest first in the cytoplasm. The position of these bands of thickened secondary wall,

which give a distinctive sculpturing to the cells of the xylem, was shown by Crüger in 1855 to follow the distribution of bands of denser cytoplasm in the differentiating cell. In this paper which has too often been overlooked by modern botanists, Crüger showed that in living cells, later destined to be ringed, spiral, or reticulate elements, actively streaming strands of denser cytoplasm occupy the positions where the lignified thickenings are to be laid down. These observations were later confirmed and extended by Dippel (1867) and Strasburger (1882) and have been discussed by other European workers. Somewhat similar results were described more recently by Barkley (1927) and Majumdar (1940). The developmental mechanism by which the different cell types (ringed, spiral, or pitted) arise thus seems quite different from that suggested by Stover (1924) who believes that the type of wall thickening in vessels is determined by the degree of elongation in the surrounding tissue in such a

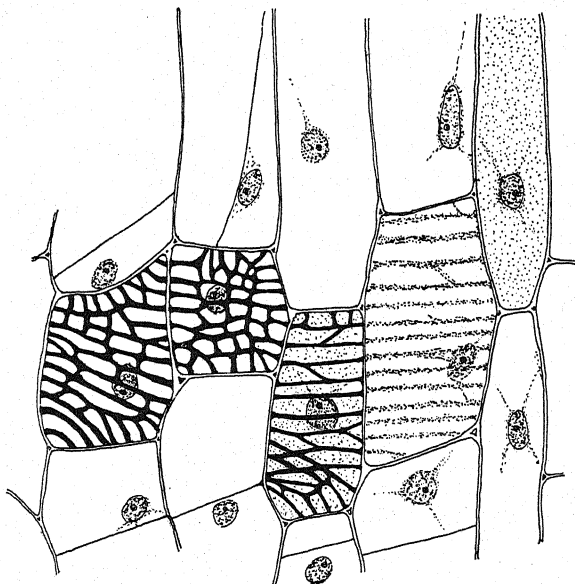


Fig. 9. Portion of a regenerating xylem strand, showing cell divisions in pith cells and ringed and reticulate lignified band pattern in various stages of differentiation. The cell at the extreme right represents an early stage showing denser cytoplasm only, while in the cell next to it bands of granular cytoplasm indicate the places where lignified thickenings will later be laid down. Camera lucida drawing. $\times 425$.

manner that the first thickening is laid down in the pitted form and that this wall thickening is torn apart and the cell becomes annular, spiral, or reticulate, depending upon the amount of stretching.

In the present material these bands of densely granular cytoplasm can readily be observed in stained preparations as the first visible steps in the transformation of a pith cell into a ringed or reticulate xylem element (fig. 9). That these markings are in the cytoplasm and not in the wall is evident from the fact that they may be observed in plasmolyzed cytoplasm, as was noted by Crüger (1855)

and Dippel (1867) (fig. 10). Within a short time after their appearance small bands of secondary wall may be demonstrated in those parts of the wall which are directly in contact with these strands. The formation of such cytoplasmic patterns was also observed by the present writers in the provascular cells of the meristem which give rise to typical protoxylem and in similar cells in regenerating bundles, but can be demonstrated much more readily in large parenchyma cells which are converted directly into banded xylem elements.

In the regeneration of phloem strands, also, the establishment of new rows of cells along paths quite independent of the original tissue axis can readily be observed and has been described by Kaan Albest (1934). These cells, cut out of the sides of pith cells, are often linked up in a zig-zag course to form a new strand (fig. 2). Here the close relationship

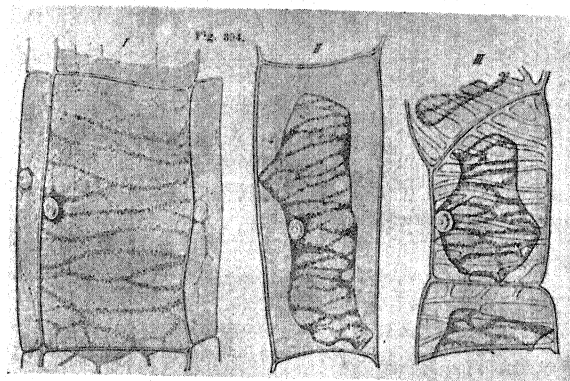


Fig. 10. Three figures from Dippel, showing development of reticulate wall thickenings in xylem cells of *Impatiens noli tangere*. Cell I shows bands of streaming cytoplasm. Cells II and III are plasmolyzed. Cell II, wall thickenings not yet developed, bands of denser cytoplasm visible in retracted protoplast; cell III, wall thickenings present, but bands still evident in cytoplasm.

of changes in one cell to those in adjacent ones is particularly evident, for each reconstructed phloem cell, laid down in a single pith cell, is directly continuous with its neighbors on either side.

DISCUSSION.—One conclusion which can be drawn from the evidence here presented is that a very early step in the redifferentiation of cells is a change in the orientation of their cytoplasmic systems. In cells which were presumptive pith parenchyma but now are destined to become vascular tissue and lie along the course where a new vascular bundle is to be reconstituted, the visible pattern of cytoplasm is reorganized to conform to the axis of the new strand rather than to the axis of the stem, as at first.

The stimulus for the development of the strand seems to originate at the basal end of the severed bundle but what determines its course through the pith to contact with some other portion of the vascular system is not clear. Evidently a particular series of cells, by virtue of its position in relation

to the severed bundle ends, the wound, and the rest of the organized system of the axis, undergoes specific changes which result in the differentiation within it of a new strand. Here there seems to be operative what is sometimes called a morphogenetic field—a series of developmental processes, governed by the positional relationships of the parts of a living system, by which a specific pattern of development is produced. In a bundle here being reconstituted the effect of this field seems first to be a radical change in the orientation of the cytoplasm of each cell concerned, a change which is soon visible in the position of new walls, and especially in the configuration of their lignified thickenings.

It is also noteworthy that these changes involve not individual cells alone but relationships between a considerable number of cells. The cytoplasmic changes which take place in one cell, as the first step in differentiation, are never independent of those which take place in neighboring cells. Thus the position of new cell walls has been shown (Sinnot and Bloch, 1941b) to be affected by the positions of similar walls in adjacent cells. In some tissues these walls are directly opposite each other so that a continuous series is formed. In others, the walls avoid each other and thus are "staggered." Since wall position is here determined by a cytoplasmic body, the phragmosome, these intercellular relations in wall position are due to relations between the cytoplasmic systems of the two cells.

In the same way, the cytoplasmic bands, in a differentiating xylem cell, which are the visible precursors of lignified thickenings, are related to similar bands in adjacent cells. This is shown not only by material like that here described but by the fact, long ago pointed out by Rothert (1899), that in adjacent ringed elements of the protoxylem the rings in one cell are directly opposite those in its neighbor (fig. 3). In reticulate and pitted elements this relationship is obvious for it results in the pits in one cell being opposite those in adjacent cells.

There are other cases where configurations in the wall form a continuous pattern extending over many cells, as in the Casparian strip of the endodermis. The remarkable "réseau de soutien" (Van Tieghem, 1888), branching bands of thickened wall extending across a whole group of cells in the cortex of gymnosperms and dicotyledons, e.g., the Cruciferae, or in the cortex of the air roots of orchids (Leitgeb, 1865; fig. 8), and the continuous thickenings in the endothecium of anthers are other examples.

What the factors are which control the formation of these banded wall patterns is not clear. Similar rhythmic patterns in animals—the stripes of the zebra, the zoned wings of butterflies, the dermal papillae on our fingers, and others—are believed by many workers to be due to essentially the same factors which produce the familiar Liesegang rings in colloidal media. The formation of lignified bands in xylem cells may result from the same causes,

and Küster (1931) has discussed the significance of the Liesegang phenomenon for such cells. Other factors are probably concerned, as well. The fact that the bands tend to assume a minimal length, which is particularly evident in their configuration at the corners of rectangular cells (fig. 6), and that their position is determined by semi-liquid strands of cytoplasm, would suggest that surface tension has a part in their distribution. At all events, it is clear that the factors responsible for pattern are operative over the living material of a considerable group of cells, and that the intercellular relationships thus established are the basis of the regular and harmonious patterns which are developed.

The differentiation of these reconstituted histological patterns in regeneration seems not to differ in its essential character from that which occurs in normal development. Indeed, there are many cases in which strands typically are differentiated in the midst of fundamental parenchymatous tissue. Thus in *Luffa* (Sinnott and Bloch, 1943), a system of interconnecting phloem fiber bundles, involving marked alterations of cells along their course, is differentiated within a homogeneous tissue of parenchyma with no regard to the original orientation and polarity of the cells, as though a new developmental pattern were superposed on an older one. This is essentially what occurs in strand regeneration as here described. Whatever can be learned from such experimentally induced differentiation should be of value for an understanding of the development of histological pattern in general.

SUMMARY

In young internodes of *Coleus* not yet fully grown, regeneration of vascular strands across par-

enchymatous tissue of the pith was induced by severing one or more large vascular bundles. The new bundle connections included both xylem and phloem strands. The former consisted in part of elongate tracheids and vessels formed by procambial divisions parallel to the course of the new strands, and in part of vacuolate and nearly mature parenchyma cells, essentially isodiametric in outline, which had differentiated directly into spiral or reticulate xylem elements without division.

Although the original axes of elongation of the pith cells are parallel to the axis of the internode, both the new divisions in them and the configuration of the lignified bands and of the pores in the undivided cells have a definite orientation relative to the axis of the new strand.

This reorientation is first evident in the cytoplasm of the cells, since the position of the new walls is preceded by a cytoplasmic diaphragm and since the position where each lignified band will later be deposited is occupied in early differentiation by a band of densely granular cytoplasm.

This cytoplasmic pattern of bands and its final manifestation in the lignified thickenings are not independent phenomena in each cell, but form a harmonious pattern over a group of cells, the elements of the pattern in one cell being directly continuous with those in adjacent ones.

The evidence suggests that histological differentiation, both normally and in regeneration, involves the establishment of such intercellular patterns or fields in the cytoplasm of groups of contiguous cells.

DEPARTMENT OF BOTANY,
YALE UNIVERSITY,
NEW HAVEN, CONNECTICUT

LITERATURE CITED

- BARKLEY, GRACE. 1927. Differentiation of vascular bundle of *Trichosanthes anguina*. Bot. Gaz. 83:173-184.
- CRÜGER, HERMANN. 1855. Zur Entwicklungsgeschichte der Zellenwand. Bot. Zeit. 13:601-613; 617-629.
- DIPPEL, LEOPOLD. 1867. Die Entstehung der wandständigen Protoplasmatrömhchen. Abhandl. Naturforsch. Gesellsch. Halle 10:53-68.
- FREUNDLICH, H. F. 1909. Entwicklung und Regeneration von Gefäßbündeln in Blattgebilden. Jahrb. Wiss. Bot. 46:137-206.
- KAAN ALBERT, ANITA VON. 1934. Anatomische und physiologische Untersuchungen über die Entstehung von Siebröhrenverbindungen. Zeitschr. f. Bot. 27:1-94.
- KÜSTER, ERNST. 1931. Ueber Zonenbildung in kolloidalen Medien. Beiträge entwicklungsmech. Anat. Pflanz. Heft 1. 2. Aufl. Jena.
- LEITGE, H. 1865. Die Luftwurzeln der Orchideen. Denkschriften K. Akad. Wiss. Wien. Math.-Nat. Classe. 24:179-222.
- MAJUMDAR, G. P. 1940. The development and structure of protoxylem vessels in *Heracleum sphondylium* L. Proc. Leeds Phil. Soc. (Sci. Sec.) 3:642-651.
- ROTIERT, WLADISLAW. 1899. Ueber den Bau der Membran der pflanzlichen Gefäße. Abhandlungen Akad. Wiss. Krakau. Math.-Naturw. Classe 34:433-492. (Polish title and text. For résumé in German (without the plates) see Anzeiger Akad. Wiss. Krakau January, 1899, pp. 15-53.)
- SIMON, S. 1908. Experimentelle Untersuchungen über die Entstehung von Gefäßverbindungen. Ber. Deut. Bot. Ges. 26:364-396.
- SIMON, S. V. 1930. Transplantationsversuche zwischen *Solanum melongena* und *Iresine Lindeni*. Jahrb. Wiss. Bot. 72:137-160.
- SINNOTT, EDMUND W., AND ROBERT BLOCH. 1941a. Division in vacuolate plant cells. Amer. Jour. Bot. 28:225-232.
- , AND —. 1941b. The relative position of cell walls in developing plant tissues. Amer. Jour. Bot. 28:607-617.
- , AND —. 1943. Development of the fibrous net in the fruit of various races of *Luffa cylindrica*. Bot. Gaz. 105:89-99.
- STOVER, E. L. 1924. The vascular anatomy of *Calamovilfa longifolia*. Ohio Jour. Sci. 24:169-179.
- STRASBURGER, EDUARD. 1882. Ueber den Bau und das Wachstum der Zellhäute. Jena.
- TIEGHEM, PH. VAN. 1888. Sur le réseau de soutien de l'écorce dans la racine. Ann. Sci. Nat. Sér. 7. Bot. 7:375-378.
- VÖCHTING, HERMANN. 1892. Über Transplantation am Pflanzenkörper. Untersuchungen zur Physiologie und Pathologie. Tübingen.

THE EXISTENCE OF PHYSIOLOGICAL STRAINS IN *PHYSARUM POLYCEPHALUM*¹

William D. Gray

BRANDZA (1927), working with myxomycete plasmodia which he had collected from their natural habitats, observed that frequently when two plasmodia of the same species were placed in close proximity, they would not fuse but withdrew from the vicinity of each other and formed their fruiting structures separately. This separation occurred particularly when the plasmodia were of slightly different color and seemed to bear out Brandza's statement that several of the species with which he worked were each composed of different physiological strains as evidenced by their inability to fuse. Brandza's statement may be criticized, however, on the grounds that while he attempted to apply Torrend's (1907) species criterion, he had not met all of the requirements set forth by Torrend, who specifically states that the plasmodia should be grown under the same environmental conditions. One might suppose that it was quite obvious that in some of the species he mentioned, Brandza was dealing with different strains because the plasmodia were different in color; however, his own earlier work (Brandza, 1926) and the work of Kambly (1939) would cast some doubt on the advisability of employing plasmodial color either as a taxonomic character or as a factor in the delimitation of strains or races. The fact that Brandza observed that plasmodia of the same species which were slightly differently colored would frequently not fuse does not necessarily mean that the two plasmodia represented different strains but may mean that the plasmodia were in different physiological states at the time they were placed together. This is particularly plausible in view of the work of Seifriz and Zetzmänn (1935) who demonstrated that a natural pH indicator exists in the plasmodium of *Physarum polycephalum* Schw., and showed that in the course of its development, the plasmodium of this species may change color several times.

Skupienski (1934), investigating temperature relations of *Didymium xanthopus* (Ditm.) Fr., found that the typical form of this species would not produce plasmodia and fruiting bodies above 25°C. whereas another form of the same species would produce plasmodia and fruiting bodies at temperatures as high as 30°C.; Skupienski assigned the race name "*thermophilum*" to the form which was able to grow at the higher temperatures.

On the basis of the work of Skupienski and Brandza it seems possible that physiological strains do

exist in some species of myxomycetes; however, the work of Winer and Moore (1941) would tend to raise further doubt as to whether Brandza was actually working with distinct strains. These investigators, working with *Physarum polycephalum* Schw., found that when two sub-cultures from the same plasmodium were placed on different media (oatmeal agar and rice agar), after three weeks bits of plasmodia from the two different cultures would not fuse. They suggest that, because of the difference in nutrient materials with which the two plasmodia were supplied, changes were induced in the plasmodia which made them no longer compatible. Therefore, since Brandza did not actually culture the plasmodia which he tested, but collected them from the substratum on which they naturally occurred, it is altogether possible that the inability to fuse which he observed was due to differences in nutrients on which the plasmodia had fed. Using Brandza's fusion test and taking into account the findings of Winer and Moore, it should be possible to demonstrate whether or not physiological strains do exist.

It was first noticed by the writer in 1938 that two plasmodia of *Physarum polycephalum* Schw. of different origin would not fuse, even though both plasmodia were of the same age and had been fed on the same nutrient material (rolled oats, in moist chamber culture) for several months. One of these cultures had been obtained by growing plasmodia from spores collected by the writer in southern Indiana; the other had been obtained by sowing spores from sporangia collected by Mr. Robert Hagelstein on Long Island. From these preliminary observations it seemed quite likely that, since the two non-fusing plasmodia originated in widely-separated places, there might exist geographical races of this species which are so different that their plasmodia will not fuse.

MATERIALS AND METHODS.—Throughout the course of this work plasmodia of *Physarum polycephalum* Schw. were used. Stock cultures were maintained in the active vegetative state on oatmeal agar, but the plasmodia used for testing were grown by the moist chamber method of Camp (1936), since it was relatively easy to obtain small, discrete plasmodial strands by picking them from the surface of the water in the moist chamber. When testing was in progress, fresh moist chamber cultures were prepared every five or six days, and all cultures were fed only rolled oats, feedings being made at twenty-four-hour intervals. For testing the various cultures small bits of plasmodia were used, twenty-four hours after their last feeding. Cultures were tested by pairing bits of plasmodia on 3 per cent agar in petri plates. In most cases this was done by placing a 7–10 mm. length of a single plasmodial strand on the agar and then laying a similar piece of plasmo-

¹ Received for publication December 22, 1944.

This work was initiated during the writer's tenure as a National Research Council Fellow (University of Wisconsin, 1938–39). The writer is indebted to Dr. William Seifriz, Dr. G. W. Martin, Dr. B. J. Luyet, and Dr. A. R. Moore, who kindly supplied him with their cultures of *Physarum polycephalum*. Thanks are also due Dr. Martin for his helpful criticisms of the manuscript.

dium from another culture on top of it, thus forming a cross; however, in some instances larger pieces of plasmodia were used. At least three such plates were prepared for each pairing.

A number of different cultures of *P. polycephalum* were on hand at the beginning of the experimentation. These were obtained from various investigators and are herein designated by letters and numbers assigned by the writer. The cultures tested were as follows:

A—Received from Dr. A. R. Moore, Department of Psychology, University of Oregon, March 15, 1944.

The culture was received on an agar slant on which it was growing in conjunction with *Torula aestotiana*.

M-1—Received from Dr. G. W. Martin, University of Iowa, September, 1943. This plasmodium was collected in Iowa City on the hymenium of a sporophore of *Schizophyllum*.

M-2—Received in agar slant culture from Dr. Martin, March, 1944. This plasmodium was collected from rotten wood about twelve miles west of Iowa City in October, 1943.

L-1—Received September 16, 1943, from Dr. B. J. Luyet, St. Louis University. The culture was received in sclerotial form, the sclerotium having been prepared July 15, 1943, from a plasmodium kept for thirty-four months in the plasmodial stage.

L-2—Received in sclerotial form from Dr. Luyet, September 16, 1943. The sclerotium had been formed on September 10, 1943, from a plasmodium kept for three years in the vegetative state.

L-3—Received from Dr. Luyet, September 16, 1943. No notes except that the sclerotium had been formed on June 25, 1943.

S-1—Received in sclerotial form from Dr. William Seifriz, University of Pennsylvania, on September 17, 1943. No notes.

S-2—Received in agar slant culture from Dr. Seifriz, September 27, 1943. No notes.

RESULTS.—The first experiment conducted with each culture was designed to determine whether pieces of plasmodium from the same culture would always fuse. Repeated pairings in which all of the above cultures were tested have demonstrated that if two pieces of plasmodium from the same culture are placed together under the conditions described above, they will always fuse and form a single plasmodium. In the many pairings of this type involving the above eight plasmodia, failure to fuse was never observed to occur.

In subsequent experiments every culture was paired with each of the other cultures. Results obtained from these pairings are shown in table 1.

From the results of table 1, it is evident that, based upon whether or not fusion of paired plasmodia occurred, three distinct plasmodial strains or races occur among the eight different cultures of *P. polycephalum*. The first consists of a single culture (*A*); the second consists of five cultures (*L-1*, *L-2*, *L-3*, *S-1*, *S-2*); the third consists of the *M-1* and *M-2* cultures. It is interesting to note that all of the *L* and *S* cultures fuse, regardless of the combination in which they are paired; however, this is not surprising in view of the fact that Dr. Luyet in-

TABLE 1. Results of pairing plasmodial strands from cultures *A*, *M-1*, *M-2*, *L-1*, *L-2*, *L-3*, *S-1*, and *S-2*. At least three pairings were made for each combination. ("+" indicates that fusion occurred; "—" indicates that fusion did not occur.)

	A	L-1	L-2	L-3	M-1	M-2	S-1	S-2
A	+	—	—	—	—	—	—	—
L-1	—	+	+	+	—	—	+	+
L-2	—	+	+	+	—	—	+	+
L-3	—	+	+	+	—	—	+	+
M-1	—	—	—	—	+	+	—	—
M-2	—	—	—	—	+	+	—	—
S-1	—	+	+	+	—	—	+	+
S-2	—	+	+	+	—	—	+	+

forms me that he originally obtained his culture from Dr. Seifriz.

The fact that cultures *M-1* and *M-2* proved to be the same strain is interesting since they were collected in the same general region. Testing of a large number of cultures from different regions may reveal that a large number of geographical strains exist or may yield some definite pattern of distribution of the various strains.

Since, in the testing of the eight cultures listed above, evidence of the existence of three strains was obtained, it was thought that further proof could be obtained if three plasmodia emerged from a culture prepared by placing strands from all eight cultures together. Accordingly, an experiment was set up in which small strands from all eight cultures were placed on agar in the same petri dish. The strands were so arranged that all of their centers crossed, so that when the inoculations were complete the eight plasmodial strands formed a sixteen-pointed star in the center of each plate. The results that were obtained were in accord with what might have been predicted in that three distinct plasmodia resulted in each plate: one fairly large, since presumably it resulted from the fusion of five pieces (*L-1*, *L-2*, *L-3*, *S-1*, *S-2*); a second quite small (probably *A*); and a third which was intermediate in size, the assumption being that it resulted from the fusion of two pieces (*M-1* and *M-2*).

Within certain limits, the difference in ages of two plasmodial strands (time interval between sub-culturing and pairing) has no bearing on whether or not the paired plasmodia will fuse. Using culture *M-1*, three day old plasmodia readily fused with four, five, and six day old plasmodia. Obviously, however, as already pointed out by Brandza, if one of the two plasmodia is advanced to the stage where it is about to form fruiting bodies, no fusion would be likely to occur.

Nauss (1943) reported that in culture the plasmodium of *Hemitrichia vesparium* (Batsch) Macbr. may be parasitized and completely consumed by a white plasmodium which she has not identified. Hagelstein (1936) reported that when two plasmodia (one green, the other yellow) were placed to-

gether, they fused and the next day the yellow one had disappeared, probably devoured by the green plasmodium. In no instance in the present work was any plasmodium observed to be consumed by another.

DISCUSSION.—It is evident, from the results presented above, that the species *Physarum polycephalum* Schw. is composed of a number of races or strains, the plasmodia of which are incompatible to the extent that they will not fuse. It should be noted that this incompatibility in no way approaches the type of antagonism exhibited by some of the filamentous fungi, different strains of which are antagonistic to the degree that when in paired culture the two mycelia will not grow within a certain distance of each other. In the case of two different strains of *P. polycephalum*, when bits of plasmodia are so placed that they touch each other, they will not fuse but will move away to different parts of the petri dish; however, in the course of the next three or four days they may touch or overlap each other many times. When such plasmodia are in contact, microscopic examination reveals that they are not fused, but the outline of each plasmodium may be followed and is seen to be distinct, with the veins of one crossing the other but never fusing. It is evident that each strain is physiologically distinct and different enough from the others to prevent fusion of the plasmodia. Seifriz (1944) has noted that sometimes plasmodia of this species will not fuse, and this investigator attributes lack of fusion to the presence of exotoxins exuded by the plasmodium. He states that if two plasmodia approach each other rapidly, they will always fuse since there has been no time for the accumulation of waste products on the approaching surfaces of the plasmodia; however, if the approach is gradual, allowing waste products to accumulate, no fusion takes place. In the present work, failure to fuse can scarcely be attributed to exotoxins because in all of the pairings that were made, the plasmodial strands were taken from the surface of the water of the moist chamber cultures, and if any exotoxin had been exuded it would have probably diffused into the water. Furthermore, no time was allowed for the accumulation of exotoxins, since when the pairings were made the plasmodia were taken from moist chamber cultures and immediately placed in direct contact with each other. Observations made by the writer on pairings of two plasmodial strands of the same strain do not tend to support the hypothesis that fusion was prevented by exotoxins, since in a number of such cultures actual fusion did not occur for several days—ample time for the accumulation of exotoxins.

Particular attention is called to the fact that *L* and *S* cultures fused, which fact indicates that these cultures belong to the same strain. On the one hand this is not surprising in view of their common origin; however the fact that they maintained their strain identity after a number of years of handling in different laboratories, by different investigators, probably under somewhat different environmental condi-

tions, would indicate that there is a certain constancy in the physiological attributes of the strains. It is not surprising that different strains of this species have arisen, since plasmodia are admirably adapted for influence by many factors of their environment; however, the fact that the *L* and *S* cultures have maintained their common identity serves to demonstrate that strain distinction is more deeply seated than might be supposed. Winer and Moore have demonstrated that what might be termed a "false strain"² may be induced by using different nutritive materials, and further investigation may reveal that different strains may be produced by altering certain other of the environmental conditions. This has not been demonstrated, however, except in the case of nutrients.

The testing of a large number of cultures from widely separated localities may yield information relative to geographical races of this species and may also yield a pattern of distribution which might be of some ecological significance.

Perhaps the most significant result of these findings is the fact that it makes easily available different strains of an organism which lends itself easily to physiological and physico-chemical investigations on practically pure protoplasm as is evidenced by the work of Seifriz (1936), Luyet and Gehenio (1942), Moore (1935), Brooks (1925) and others. Careful investigation of different strains of this organism may yield information of fundamental importance for the explanation of slight differences between closely-related protoplasms.

SUMMARY

Small plasmodial strands from eight cultures of *Physarum polycephalum* were paired in all possible combinations. Fusion occurred consistently in some combinations; never in others. On the basis of this reaction the eight plasmodia could be divided into three strains.

Plasmodia from the same original source but maintained in different laboratories by different investigators for several years retained the capacity to fuse.

Some evidence was found indicating the existence of geographical strains in this species.

There was no evidence of the prevention of fusion by the formation of exotoxins.

BIOLOGICAL LABORATORY,
U. S. QUARTERMASTER DEPOT,
JEFFERSONVILLE, INDIANA

LITERATURE CITED

- BRANDZA, M. 1926. Sur la polychromie des myxomycètes vivant en plein soleil. *Compt. Rend. l'Acad. Sci., Paris* 182: 987-989.
———. 1927. Sur la fusion ou la separation des plasmodiums comme criteriums dans la definition de l'espèce chez les myxomycètes. *Compt. Rend. l'Acad. Sci., Paris* 185: 1072-1074.

² This writer's designation.

- BROOKS, S. C. 1925. The electrical conductivity of pure protoplasm. *Jour. Gen. Physiol.* 7:327-330.
- CAMP, W. G. 1936. A method of cultivating myxomycete plasmodia. *Bull. Torrey Bot. Club* 63:205-210.
- HAGELSTEIN, R. 1936. On preparing an exhibit of the life cycle of the mycetozoa. *Jour. New York Bot. Gard.* 37:140-145.
- KAMBLY, PAUL E. 1939. The color of myxomycete plasmodia. *Amer. Jour. Bot.* 26:386-390.
- LUYET, B. J., AND P. M. GEHENIO. 1942. The problem of the response of mycetozoa to gravity. *Biodynamica* 4:1-32.
- MOORE, A. R. 1935. On the significance of cytoplasmic structure in plasmodium. *Jour. Cell. and Comp. Physiol.* 7:113-129.
- NAUSS, RUTH N. 1943. Observations on the culture of *Hemitrichia vesparium*, with special reference to its black plasmodial color. *Bull. Torrey Bot. Club* 70:152-163.
- SEIFRIZ, WILLIAM. 1936. Reaction of protoplasm to radium radiation. *Protoplasma* 25:196-200.
- . 1944. Exotoxins from slime molds. *Science* 100:74-75.
- , AND M. ZETZMANN. 1935. A slime-mould pigment as indicator of acidity. *Protoplasma* 23:175-179.
- SKUPIENSKI, F. X. 1934. Sur l'existence de races physiologiques chez les myxomycetes. *Annales de Protistologie* 4:121-132.
- TORREND, C. 1907. Les myxomycetes. *Étude des espèces connues jusqu'ici*. *Broteria* 6:5-64.
- WINER, B. J., AND A. R. MOORE. 1941. Reactions of the plasmodium *Physarum polycephalum* to physico-chemical changes in the environment. *Biodynamica* 3:323-345.

THE PRODUCTION AND CHARACTERIZATION OF ULTRAVIOLET-INDUCED MUTATIONS IN *ASPERGILLUS TERREUS*. I. PRODUCTION OF THE MUTATIONS¹

Alexander Hollaender, Kenneth B. Raper, and Robert D. Coghill

IN PREVIOUS publications, Hollaender and Emmons (1939) and Emmons and Hollaender (1939) have described the lethal and sublethal (including genetic) effects of monochromatic ultraviolet radiation on *Trichophyton mentagrophytes*. In that work it was found that wavelengths between 2000 and 3000 Å were highly effective in the inactivation of the organism. Similar reports have been given by other investigators on a number of other fungi (Landen, 1939; Zahl, Koller, and Haskins, 1939; Dimond and Duggar, 1940). It was also found that the wavelength which is most effective in killing is the same wavelength which is most efficient in the production of mutations, i.e., 2650 Å. In other publications the effectiveness of wavelengths longer than 3300, which produce toxic effects at very high energy values, were described (Hollaender, 1943). However, at wavelengths longer than 3300 no genetical effects have been found.

The mutations reported on *Trichophyton* were "morphological"; they differed from the normal in rate of growth, colony form, spore formation, mycelium formation, pigment production, and in other characteristics. These changes in the appearance of the organism persisted for many transfers. Since *Trichophyton mentagrophytes* has no known sexual stage, it was not possible to analyze the mutations by accepted genetical techniques. A similar study with *Neurospora crassa*, a fungus with a sexual stage, permitted Hollaender, Sansome, Zimmer, and Demerec (1945) (see also Lindegren and Lindegren, 1941) to make a genetical analysis of the mutations produced by radiation. It is therefore quite safe to state by analogy that the changes produced in *Trichophyton* are definitely genetical. Mutations produced by ultraviolet have been studied by other investigators including: Stadler and Ueber

(1942) on the pollen of maize; Knapp, Reuss, Risse, and Schreiber (1939) on liverwort; and Mackenzie and Muller (1940) and Demerec, Hollaender, Houlahan, and Bishop (1942) on *Drosophila*. These studies have well established the fact that ultraviolet is an efficient means of producing mutations.

There is little doubt that the change in morphological appearance of the fungus colonies is based on a change in the chemical makeup of the irradiated spores or in a change in the succession of chemical events in the irradiated spores. It is reasonable to expect that changes in the genetical makeup of an organism would interfere with its metabolic function by destroying certain enzymes, which might make it impossible for the organisms to produce certain vitamins, enzymes, amino acids, etc., essential for the organism if it is to grow on a simple synthetic medium. The technique showing this effect has been established by the group working with Beadle and Tatum (1941). Another possibility is that the irradiation produces a genetical change resulting in a change of chemical function, which brings about an increase in the yield of certain chemicals produced by the organism. It is conceivable (1) that the chemicals are produced as byproducts or waste products and are of no further use to the organism, or (2) that they are intermediates from which the organism can synthesize certain essential compounds. It should be possible, therefore, to shift the metabolism of an organism by changing its genetical makeup, so that the production of these waste materials or intermediate metabolites is decreased or increased. The production of a mutation with increased yields of waste products might be a rarity, but the possibility definitely exists. In general, in attempting to make an organism produce appreciable quantities of a certain chemi-

¹ Received for publication December 30, 1944.

cal, one starts out with a strain which is known to produce this substance at least in a small quantity.

We came across this type of phenomenon in colonies which originated from irradiated spores of *Trichophyton*. It was noticed that the normal organism produced a small amount of pigment. Occasionally mutations were found which produced similar types of pigment in large quantities. These mutations produced sufficient pigment to permit its isolation. Kresling and Stern (1936) reported that the production of citric acid was increased in a radon-induced mutation of *Aspergillus niger*.

The technique of modifying the metabolic action of micro-organisms is of considerable promise and has many applications. In this series of articles we shall describe a preliminary study of the effects of monochromatic ultraviolet radiation on *Aspergillus terreus*. The first article will describe the physical aspects of this problem; the second will discuss the mycological aspects and describe mutations with certain deficiencies; and the third will describe itaconic acid production by organisms coming from the irradiated and nonirradiated control spores. The strain of *Aspergillus terreus* which was used in this investigation represented the best itaconic acid producer among some 30 cultures studied by Moyer and Coghill (1945). The purpose of our experiments was to produce mutations which would yield sufficient itaconic acid to make their industrial use feasible. This work was done in 1941. Because of certain war exigencies, publication has been delayed until now.

The culture of *Aspergillus terreus* used in our tests was No. 265 from the culture collection of the Northern Regional Research Laboratory, and was earlier obtained from the Thom collection as No. 5474.F11. The organism was originally isolated from Texas soil by Professor Marie B. Morrow (ca. 1936). Cultures seeded upon Czapek's solution agar slants were incubated for 10 days at room temperature. Growth was luxuriant and spore production heavy, the cultures becoming cinnamon brown in six to eight days from the production of

abundant conidial heads. The spores (conidia) were washed off in sterile water containing lauryl sulfonate in a concentration of 1 to 100,000, the suspension was shaken in a culture tube vigorously by hand, filtered through sterile absorbent cotton, and precipitated by centrifugation. The precipitated spores were resuspended in sodium lauryl sulfonate and refiltered; the resulting suspensions were quite uniform and were used for irradiation in a concentration of about 100×10^6 spores per cc. The number of spores was determined in a blood counting chamber.

IRRADIATION TECHNIQUE.—Radiation from a medium-pressure, water-cooled, quartz-capillary, mercury-vapor lamp of the Daniels-Heidt type was concentrated on the entrance slit of a large quartz monochromator. The emerging monochromatic beam was concentrated on a standardized thermopile or on the front face of a special exposure cell. The material in the exposure cell was stirred during irradiations so that on the average each spore was exposed to an equivalent amount of energy. Before and during the process of irradiation samples were removed for plating. (For details, see Hollaender and Claus, 1936).

This study covers three wavelengths: 2280 Å, 2650 Å, and 2967 Å. It has been customary in our laboratory to use a larger number of wavelengths; but since our purpose in this study was to get sufficient material for testing the effect of ultraviolet irradiation on itaconic acid production, only a limited number of wavelengths were used. The purity of these bands was fairly high. All energy entering the exposure cells was absorbed because the density of the suspension was so high that none of the radiation penetrated the entire cell.

TREATMENT AFTER IRRADIATION.—After irradiation the spore suspension was diluted with physiological salt solution and plated in Czapek's solution agar. At least three plates were poured for each dilution, and usually two or three dilutions were made. The colonies were counted after an incubation period of 3 to 4 days at 26°C. All the colonies were

TABLE 1. Energy absorption, survival, and mutation data from irradiation of *Aspergillus terreus* in a typical experiment (exp. 29) using 2650 Å.

Run	Control	1	2	3	4	5	6	7	8	9	10
Energy (absorbed) in ergs per spore 10^{-4} ...	0	12.5	25.2	43.9	61.6	81.9	112.2	131.8	160.3	214.3	248.6
No. of viable spores remaining ^a	100×10^6	65×10^6	64×10^6	14×10^6	2.5×10^5	6.7×10^5	2.7×10^5	6.2×10^4 ^b	2.3×10^4 ^b	1.1×10^4 ^b	6.4×10^3 ^b
Per cent survival	100	65	64	14	2.5	.67	.27	.062	.023	0.014	.0065
No. of colonies isolated	30	30	30	30	30	30	30	30	30	30	30
Per cent mutation	0	13.4	13.4	26.7	26.7	6.8	10	3.0	6.8	13.6	10

^a Spores plated out immediately after irradiation. For details see text. Values marked (b) are the less reliable ones.

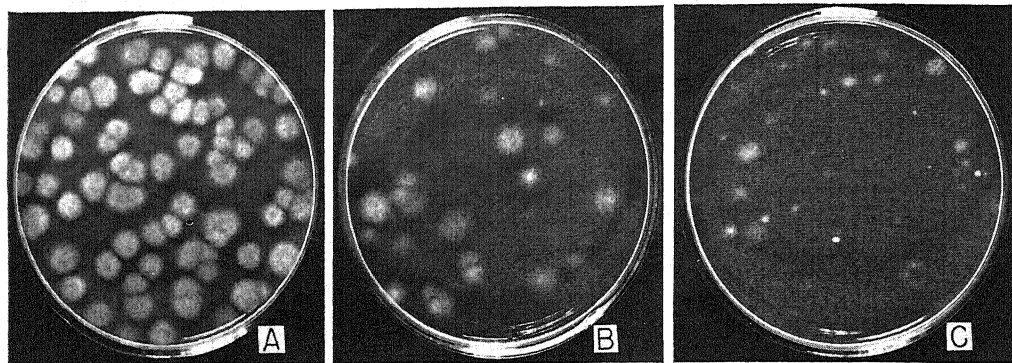


Fig. 1. *A*, Control colonies developing from non-irradiated conidia; dilution 1:1,000,000; age, 3 days. *B*, Irradiation at 2967 Å for 1 hour; dilution 1:1,000,000; age, 4 days. *C*, Irradiation at 2967 Å for 2 hours; dilution 1:100,000; age, 4 days. Note progressive reduction in number of colonies developing and decrease in growth rate of individual colonies.

counted on plates containing from 20 to 200 colonies per plate. In general, the irradiated spores germinated or formed visible colonies considerably later than the controls. This is illustrated in figure 1. After the colonies were counted, certain sections of the control and irradiated plates were marked off, and single individual colonies were transferred to Czapek's solution agar slants. The mutation rates were determined by examination and comparison of these tube colonies 6 to 10 days after transfer.

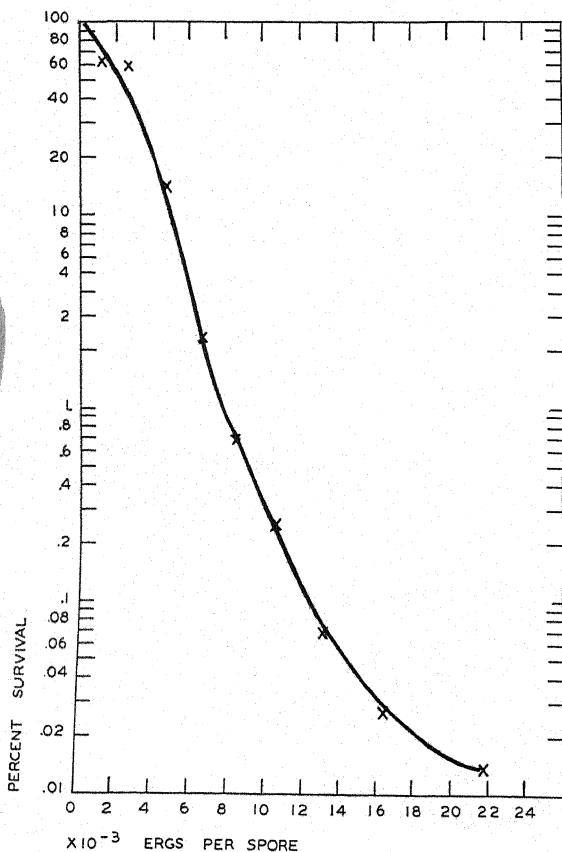


Fig. 2. Per cent survival plotted against energy absorbed per spore (λ 2650 Å).

RESULTS.—The results of a typical experiment are given in table 1. The energy is given in ergs absorbed per spore. For the method of calculating the energy, see Hollaender and Emmons (1939). The number of viable spores was determined from plate counts, and the values are averages of at least three plates. The data on runs 7, 8, 9, and 10 are less reliable. In figure 2 we have plotted the survival ratios against energy absorbed per spore.

Table 2 shows the energies necessary to produce 50, 75, 90, 99, and 99.9 per cent killing for the three wavelengths used. The variability of the sensitivity of the spores from batch to batch is quite striking. (See data on λ 2650 Å.) Why this is so is difficult to say; it might be caused by the differences in thickness of cell wall and the density of the pigment. Since the ultraviolet must penetrate to the chromatin before it can become effective, it is essential that it pass through the cell wall and a considerable portion of the cytoplasm. This same variation in sensitivity of different batches of spores has also been observed in other fungi (Hollaender and Emmons, 1939; Hollaender, Sansome, Zimmer, and Demerec, 1945 (in press)).

The relative sensitivity of the spores to the other wavelengths tested in these experiments falls into the general pattern observed previously, i.e., the highest sensitivity is at 2650 Å, followed by decreased sensitivity at 2280 Å, and finally by the lowest sensitivity at 2967 Å. For a detailed analysis of a typical fungus inactivation curve see Hollaender and Emmons (1939).

Colonies from control and irradiated plates were isolated on Czapek's solution agar slants. No selection was made; the number usually picked was 30 per exposure, while in one case (expt. 32) only 15 colonies per exposure were isolated. These slants were incubated for 10 to 14 days at 26°C. At the end of this period, a careful examination of the isolated colonies was made, and the colonies which appeared different from the normal were separated. Since the Czapek's solution agar is an incomplete medium,² the tendency for organisms which had lost

² No attempt was made to use the most highly purified chemicals.

TABLE 2. Effectiveness of three wavelengths in killing *Aspergillus terreus* spores. All values in ergs per spore.

Wave length	Experiment number	50%	75%	90%	99%	99.9% killing
2650 Å	F29	20×10^{-4}	38×10^{-4}	68×10^{-4}	78×10^{-4}	125×10^{-4}
	F32	14	17	24	30	60
	F34	22	35	60	80	120
2280 Å	F33	84	88	100	140	..
2967 Å	F30	210	320	440	700	..

the ability to synthesize certain essential compounds would be to grow very slowly on this medium and not necessarily show the usual characteristics associated with *Aspergillus terreus*.

A classification of the mutations in a typical experiment as they appeared in the initial agar slant cultures is given in table 3. In general, this tentative grouping agrees with that subsequently developed in the repeated cultivation of mutant cultures. (For a detailed discussion of the cultural and morphological characteristics of the mutants, see the second article in the series by Raper, Coghill, and Hollaender (1945). In table 3 classifications are based only on morphological appearances and do not bear any relation to possible chemical activity. We will discuss here only the quantitative aspects of the appearance of the morphological mutations. It is important to point out that in the main these persisted through many transfers and can be accepted as permanent (Raper, Coghill, and Hollaender, 1945). It should be kept in mind that the colonies were grown on Czapek's solution agar. When the cultures were transferred to a more complete medium (e.g., potato-dextrose or malt agar) many more of the cultures appeared normal, and the percentage of morphological mutations was materially reduced.

The survival ratio curve and the corresponding mutation curve for λ 2967 Å are plotted in figure 3. The percentage mutation refers to the number of colonies from irradiated surviving spores which showed marked morphological changes. As Emmons and Hollaender (1939) and Hollaender and Em-

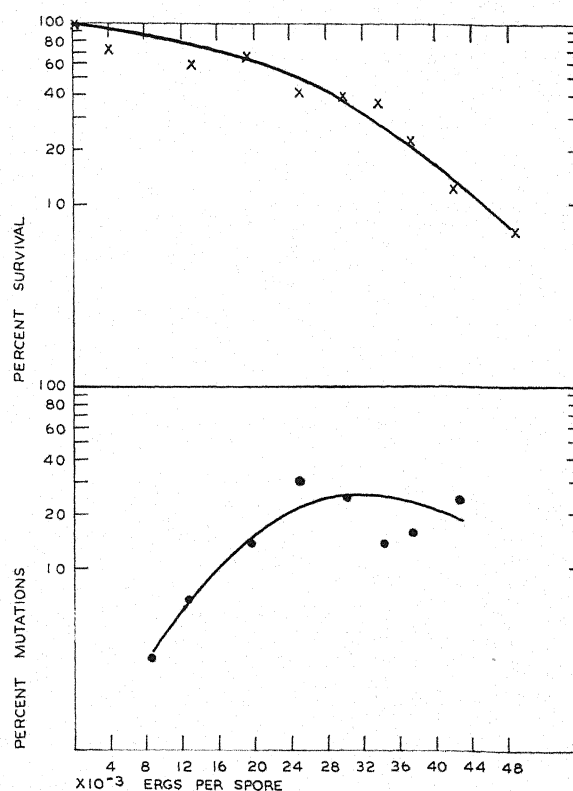


Fig. 3. Upper graph: per cent survival against energy absorbed per spore.—Lower graph: per cent mutations against energy absorbed per spore for corresponding survivals as given in upper graph.

TABLE 3. Numbers and types of mutations obtained in a typical experiment (exp. 29) using 2650 Å.

Colony type	Sample number										
	Control	R-1	R-2	R-3	R-4	R-5	R-6	R-7	R-8	R-9	R-10
1. Normal appearing—red exudate.....	1	1	..	1	1
2. Very woolly—dark diffuse pigment.....	..	1	1	1	..
3. Yellow woolly	1	..	2	1	1	..
4. White woolly	1	1
5. Slow-growing—small	1	1
6. Limited colony—uniform appearing.....	1	..	1	3	..	1	1
7. Limited colony—raised, late sporing....	1	2	2	2	1	1	1	1	1	1	1
8. Leathery	1
9. Erupted	1	..	1
10. White conidial heads.....	1

TABLE 4. Per cent survival and per cent mutations resulting from irradiated conidia when plated out immediately after irradiation and after incubation for 2 and 4 days in physiological salt solution. Each figure is based on 15 isolations made at random. Irradiation with 2650 Å.

	Exposure time (minutes)	Per cent survival	Per cent mutations after incubation		
			0 days	2 days	4 days
Control	0	100	0	0	0
Run 1	15	74	13.3	26.6	33.3
Run 2	25	6.4	20	?	47
Run 3	41	.7	13	13.3	13.3
Run 4	61	.09	19	33.3	13.3

mons (1941) observed previously, the mutation curve rises to a maximum and then declines rather slowly or else becomes more or less erratic. However, the number of colonies isolated in this study was too small to permit of a more careful analysis of this phenomenon.

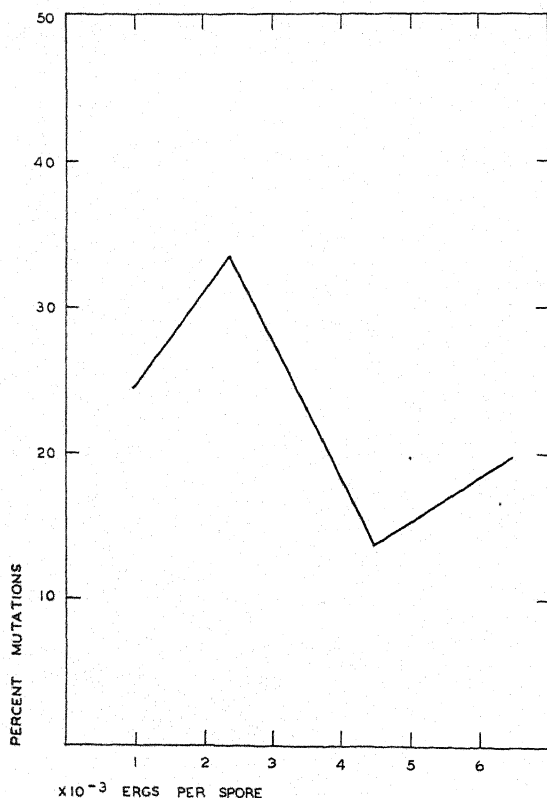


Fig. 4. Per cent mutations (summary of mutation rates given in table 4) against energy absorbed per spore.

It had previously been observed that incubation in certain salt solutions of the spores after irradiation might increase the percentage of mutations (Hollaender and Emmons, 1941). An extensive experiment of this sort was conducted on *Aspergillus terreus*. The organism was plated out just before and immediately after irradiation. The irradiated and control spore suspensions were then incubated

for two and four days and were plated out at the end of each period. The results of the mutation tests are given in table 4, and a graph of the per cent mutations against energy absorption is presented in figure 4.

Runs 1 and 2 show an increase of mutation with extended incubation. The appearance of mutations in runs 3 and 4 is erratic. For a discussion of the reasons for increase of mutation with incubation, see Hollaender and Emmons (1941). No effect of energy on the distribution of type of mutation could be recognized. One important point needs emphasis. A considerable percentage of *Aspergillus terreus* spores had mutated after only about 20 per cent of the spores had been killed by ultraviolet radiation. In contrast to this observation, significant mutation with *Trichophyton mentagrophytes* (Emmons and Hollaender, 1939), *Penicillium notatum* (Hollaender and Zimmer, 1944), and *Neurospora crassa* (Hollaender, Sansome, Zimmer, and Demerec, 1945) was found only after about 50 to 70 per cent of the spores were unable to form viable colonies after irradiation in the ultraviolet (2280 to 2967 Å).

SUMMARY

The method for the production of mutations in *Aspergillus terreus* by ultraviolet radiation is described.

The probability of producing "deficient" mutants through chemical changes and the occasional appearance of mutants having increased ability to produce certain compounds is pointed out.

Results are given on the per cent of mutation and survival after exposure to λ 2280, 2650, and 2967 Å.

The effect of secondary treatment after irradiation and the characteristic appearance of mutations after a relatively small number of spores are killed are reported.

INDUSTRIAL HYGIENE RESEARCH LABORATORY,
NATIONAL INSTITUTE OF HEALTH,
BETHESDA, MARYLAND
FERMENTATION DIVISION,
NORTHERN REGIONAL RESEARCH LABORATORY,
BUREAU OF AGRICULTURAL AND INDUSTRIAL
CHEMISTRY,
U. S. DEPARTMENT OF AGRICULTURE,
PEORIA, ILLINOIS

LITERATURE CITED

- BEADLE, G. W., AND E. L. TATUM. 1941. Genetical control of biochemical reactions in *Neurospora*. *Proc. Nat. Acad. Sci.* 27: 499-506.
- DEMEREK, M., A. HOLLAENDER, M. B. HOULAHAN, AND M. BISHOP. 1942. Effect of monochromatic ultraviolet radiation on *Drosophila melanogaster*. *Genetics* 27: 139-140.
- DIMOND, A. E., AND B. M. DUGGAR. 1940. Effects of ultraviolet radiation on the germination and morphology of spores of *Rhizopus sinuatus*. *Jour. Cell. and Comp. Physiol.* 16: 55-61.
- EMMONS, C. W., AND A. HOLLAENDER. 1939. The action of ultraviolet radiation on dermatophytes—II. Mutations induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. *Amer. Jour. Bot.* 26: 467-475.
- HOLLAENDER, A. 1943. Effect of long ultraviolet and short visible radiation (3900 to 4900 Å) on *Escherichia coli*. *Jour. Bact.* 46: 531-541.
- HOLLAENDER, A., AND W. D. CLAUS. 1936. The bactericidal effect of ultraviolet radiation on *Escherichia coli* in liquid suspensions. *Jour. Gen. Physiol.* 19: 753-65.
- , AND C. W. EMMONS. 1939. The action of ultraviolet radiation on dermatophytes. I. The fungicidal effect of monochromatic ultraviolet radiation on the spores of *Trichophyton mentagrophytes*. *Jour. Cell. and Comp. Physiol.* 13: 391-402.
- , AND —. 1941. Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. *Cold Spring Harbor Symposia Quant. Biol.* 9: 179-186.
- , E. SANSOME, E. ZIMMER, AND M. A. DEMEREK. 1945. Quantitative irradiation experiments on *Neurospora crassa*. I. Ultraviolet irradiation. *Amer. Jour. Bot.* (In press.)
- , AND E. ZIMMER. 1944. The effect of ultraviolet radiation and X-rays on mutation production in *Penicillium notatum* (abstract).
- KNAPP, E., A. REUSS, O. RISSE, AND H. SCHREIBER. 1939. Quantitative Analyse der mutationsauslösenden Wirkung monochromatischen UV-Lichtes. *Naturwiss.* 27: 304.
- KRESLING, E., AND E. STERN. 1936. Über die Wirkung von Radium und ultravioletten Strahlen auf die Entwicklung, die biochemischen Eigenschaften und die Rassenbildung des *Aspergillus niger*. *Zentralblatt für Bakteriologie Abt. II* 95: 327-340.
- LANDEN, E. W. 1939. The spectral sensitivity of spores and sporidia of *Ustilago zeae* to monochromatic ultraviolet light. *Jour. Cell. and Comp. Physiol.* 14: 217-226.
- LINDEGREN, C. C., AND G. LINDEGREN. 1941. X-ray and ultra-violet induced mutations in *Neurospora*. *Jour. Hered.* 32: 405-412, 435-440.
- LOCKWOOD, L. B., K. B. RAPER, A. J. MOYER, AND R. D. COGHILL. 1945. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. III. Biochemical characteristics of the mutations. *Amer. Jour. Bot.* (In press.)
- MOYER, A. J., AND R. D. COGHILL. 1945. The laboratory-scale production of itaconic acid by *Aspergillus terreus*. *Jour. Amer. Chem. Soc.* (In press.)
- MACKENZIE, K., AND H. J. MULLER. 1940. Mutation effects of ultraviolet light in *Drosophila*. *Proc. Roy. Soc. London B.* 129: 491-517.
- RAPER, K. B., R. D. COGHILL, AND A. HOLLAENDER. 1945. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. II. Cultural and morphological characteristics of the mutations. *Amer. Jour. Bot.* 32: 165-176.
- STADLER, L. J., AND F. M. UBER. 1942. Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. *Genetics* 27: 84-118.
- ZAHLE, P. A., L. R. KOLLER, AND C. P. HASKINS. 1939. The effects of ultraviolet radiation on spores of the fungus *Aspergillus niger*. *Jour. Gen. Physiol.* 22: 689-98.

THE PRODUCTION AND CHARACTERIZATION OF ULTRAVIOLET-INDUCED MUTATIONS IN *ASPERGILLUS TERREUS*. II. CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF THE MUTATIONS¹

Kenneth B. Raper, Robert D. Coghill, and Alexander Hollaender

CONIDIA of a selected strain of *Aspergillus terreus* Thom, NRRL 265, were exposed to monochromatic ultraviolet radiation in an effort to obtain mutations characterized by an increased production of itaconic acid. Selection of this strain was based upon the work of Moyer and Coghill (1945), who had found it to be the most productive of some 30 strains of *A. terreus* contained in the culture collection of the Northern Regional Research Laboratory at the time the present investigations were initiated. Employing a medium somewhat different from that used by Calam, Oxford, and Raistrick (1939), who

were the first to report the production of itaconic acid by *A. terreus*, Moyer and Coghill succeeded in obtaining substantial yields of the acid with strain 265.

The radiations were performed at the National Institute of Health, Bethesda, Maryland, and methods and apparatus previously described by Hollaender and Claus (1936) and by Emmons and Hollaender (1939) were employed. Suspensions of spores (conidia) were prepared from 10- to 12-day-old cultures, and were made up in sterile water or saline (1936) with sodium lauryl sulfonate added as a wetting agent in a concentration of 1 to 100,000. The spore suspensions were contained in a glass cell, provided with quartz win-

¹ Received for publication December 18, 1944.

Presented by Dr. Alexander Hollaender at a joint meeting of the Botanical and Mycological Societies of America, Cleveland, Ohio, September 12-14, 1944.

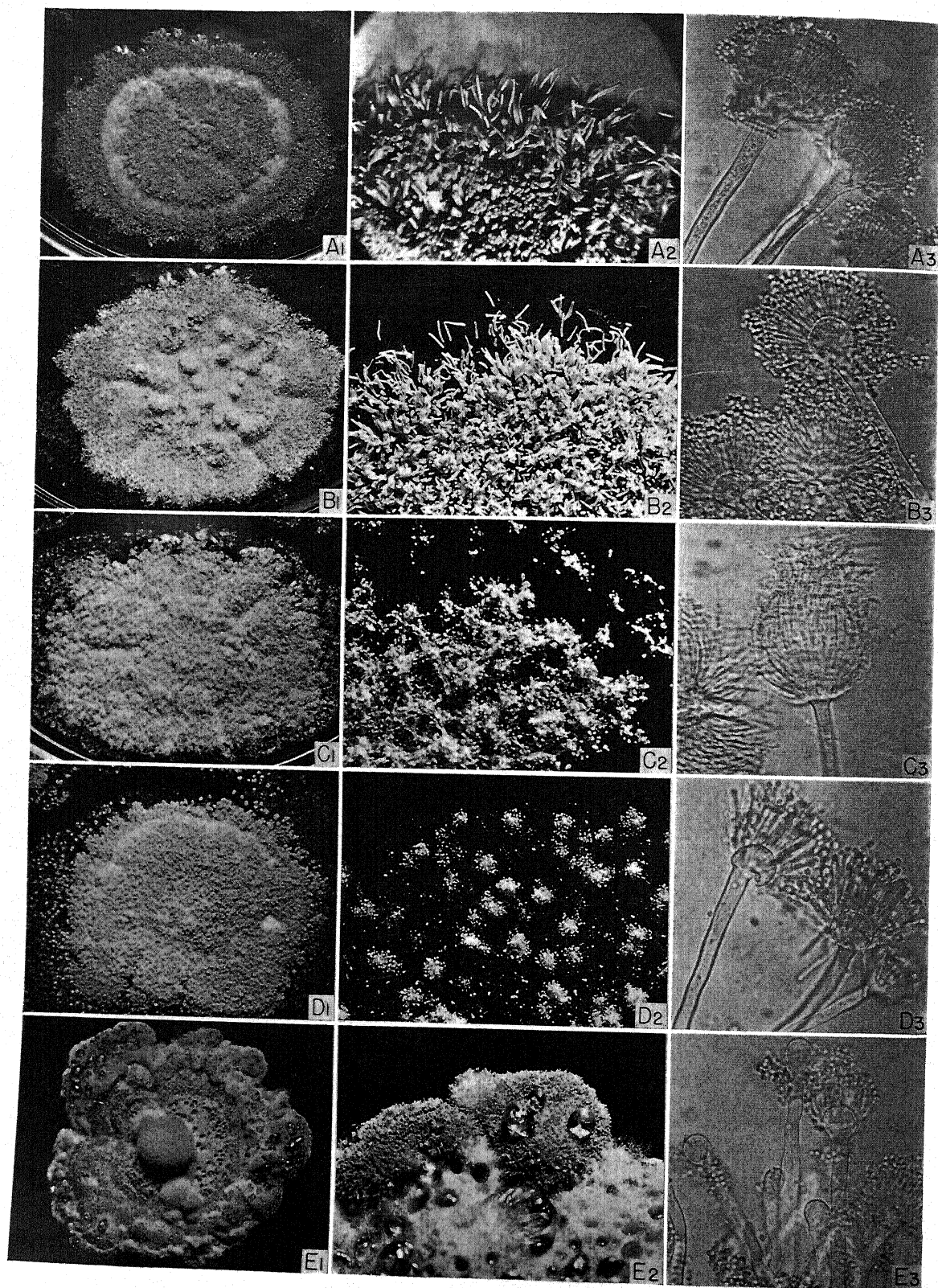


Fig. 1. Comparison of cultural and morphological characters in the parent strain and four ultraviolet induced mutations of *Aspergillus terreus*, NRRL No. 265.—A. The parent strain: A₁, colony upon Czapek's solution agar, 21

dows, during radiation and were stirred continuously to insure uniform exposure (1936). Three wave lengths were employed, namely, 2650, 2280, and 2967 Å. Samples were removed from the suspensions after different intervals of radiation and were immediately diluted and plated in Czapek's solution agar (Thom, 1930, p. 42). Following an incubation period of three to four days at 26°C., colony counts were made to determine the lethal effects of radiation; and subsequent to this, random isolations were made to determine the types and abundance of the mutants produced. Small portions of colonies from the original dilution plates were transplanted to Czapek agar slants and allowed to develop from 8 to 10 days at room temperature. The number and types of obvious mutants obtained were noted, and the cultures were retained for further study and observation. Details of the above work are reported in a separate paper by Hollaender, Raper, and Coghill (1945), which represents the first of a series of three reports dealing with the production, characterization, and evaluation of mutants of *A. terreus*.

Cultures developing from irradiated spores were investigated from two points of view. A large number of isolates, including both obvious cultural and morphological mutants and apparently normal strains, were studied in tube and Petri dish culture upon a variety of substrata over a period of 12 months to secure as much information as possible regarding the cultural, morphological, and physiological characteristics of the mutants produced. The results of this investigation are reported in the present paper. It is primarily mycological in nature and constitutes the second of the above-mentioned series. An equally large number of strains, which for the most part were the same as those included in the present study, were surveyed for the production of itaconic acid. The results of this survey are reported separately by Lockwood, Raper, Moyer, and Coghill (1945) and constitute the third paper in the series.

As a matter of convenience, results of the present study are presented under the following topics: (1) extent of study; (2) characterization of mutants; (3) high acid-producing mutants; (4) influence of the culture medium; (5) stability of mutants; and (6) general discussion.

EXTENT OF STUDY.—Altogether isolations were made from approximately 1,500 colonies resulting from irradiated spores, and it was from these isolates growing in tube cultures upon Czapek's solu-

tion agar that the data presented by Hollaender, Raper, and Coghill (1945) were compiled. In the present investigation the number of strains studied was limited to approximately 200. These included a very limited number of isolates from single, non-irradiated spores, 60 strains resulting from irradiated spores which appeared normal at the time of isolation, and approximately 130 strains resulting from irradiated conidia which appeared as obvious cultural and morphological mutants. Each of the strains was grown upon a variety of substrata, including (1) standard Czapek's solution agar with sodium nitrate and sucrose (Thom, 1930, p. 42), (2) Czapek's solution agar with ammonium nitrate instead of sodium nitrate, (3) Czapek's solution agar with urea instead of sodium nitrate, (4) Czapek's solution agar with dextrose instead of sucrose, (5) Czapek's solution agar with 1 per cent of added corn steeping liquor, (6) potato dextrose agar, and (7) malt extract agar containing two per cent dextrose, two per cent malt extract, and 0.1 per cent peptone. In addition, each of the isolates was grown through ten successive culture generations upon standard Czapek's solution agar during a period of approximately one year. The objective in employing different culture media was to determine roughly the effect that changes in substrata would exert upon the cultural and morphological appearance of the isolates. The objective in repeated cultivation upon the same medium was to determine whether altered characteristics which distinguish strains as mutants would be inherited and therefore prove stable, or whether they were transient in character and would be outbred during successive periods of growth. An even smaller number of strains, representative of the different types of mutants encountered, were studied intensively, and detailed cultural and microscopical observations were made. Characterization of the different types of mutants is based primarily upon this latter group of cultures.

CHARACTERIZATION OF MUTANTS.—Not all of the mutants produced could be readily classified, but the majority of those which were culturally apparent could be grouped into a limited number of fairly definite types. In some cases, types were represented by single isolates only. In other cases, multiple isolates belonged to the same type and closely duplicated one another in appearance, irrespective of the wave length employed or the period of radiation involved in their production. In still other cases the types were very indefinite, and while a sufficient

days, natural size; A_2 , marginal portion of colony showing crowded columnar heads, $\times 8$; A_3 , photomicrographs showing details of typical conidial heads, $\times 450$.—*B.* "Typical" albino mutant, strain 33-10-4: B_1 , colony on Czapek's solution agar, 21 days, natural size; B_2 , margin of colony showing crowded white columnar heads, $\times 8$; B_3 , conidial heads showing normal character, $\times 450$.—*C.* "Atypical" albino mutant, strain 30-9-12: C_1 , colony on Czapek's solution agar, 21 days, natural size; C_2 , marginal area showing irregularly shaped conidial heads, $\times 8$; C_3 , photomicrograph showing normal character of vesicle and sterigmata but imperfect conidium formation (see text p. 7), $\times 450$.—*D.* Granulated mutant, strain 32A-S-1-12: D_1 , colony growing upon Czapek's solution agar, 21 days, natural size; D_2 , marginal area of same colony showing aggregated conidial structures, $\times 8$; D_3 , photomicrograph showing abnormal sterigmata and few and slightly larger conidia, $\times 450$.—*E.* Yellow-white (floccose) mutant, strain 30-9-5: E_1 , colony growing upon Czapek's solution agar, 21 days, natural size; E_2 , margin of colony showing small conidial heads, $\times 8$; E_3 , photomicrograph showing abortive conidial structures consisting of vesicles devoid of sterigmata, $\times 450$.

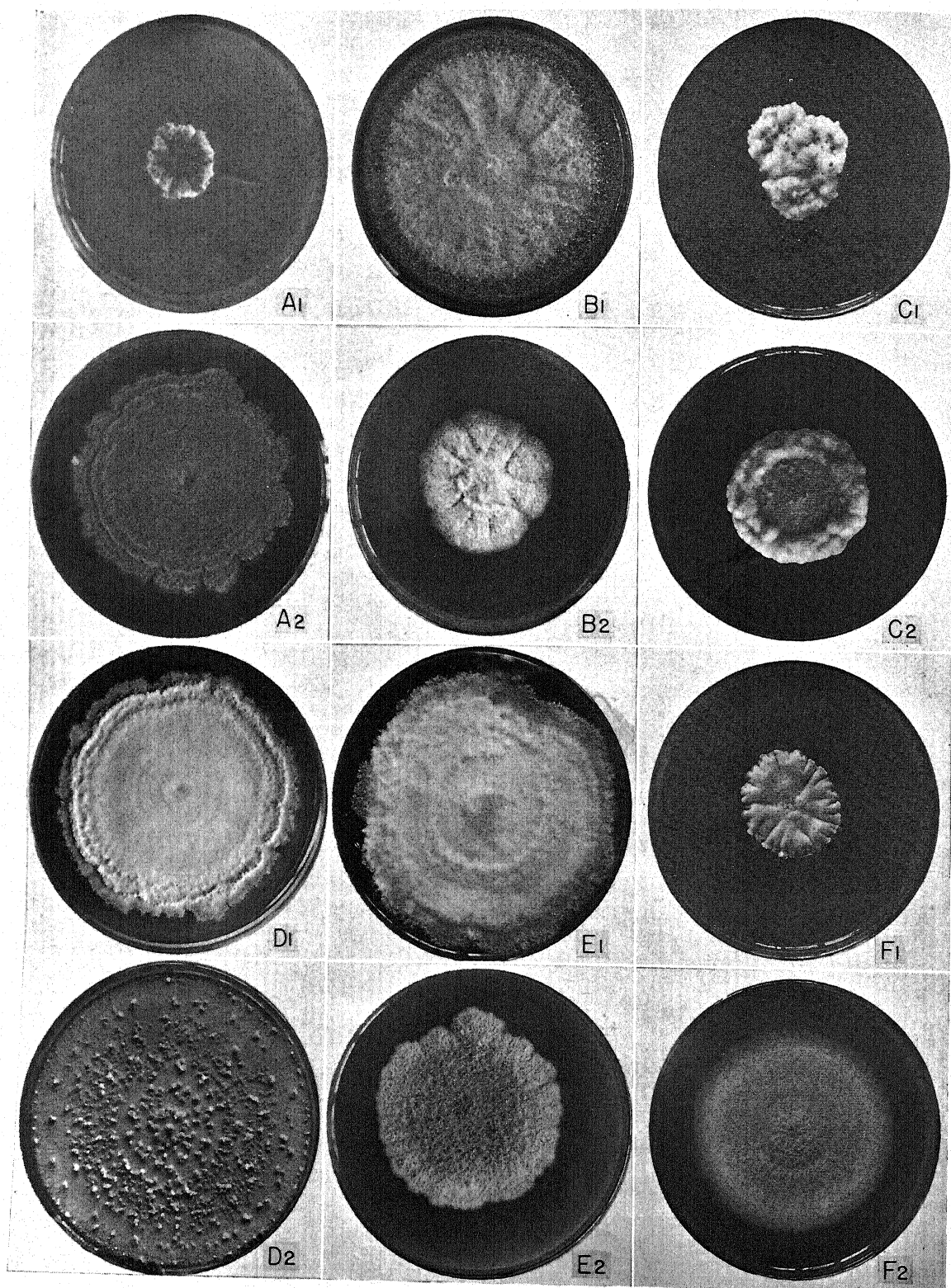


Fig. 2. Cultural and morphological mutants of *Aspergillus terreus*, NRRL No. 265, growing upon (1) Czapek's solution agar and (2) malt extract agar.— A_1 and A_2 . Mutant of type VI growing upon Czapek's solution and malt extract agars, respectively.— B_1 and B_2 . Mutant of type VII growing upon similar media.— C_1 and C_2 , D_1 and D_2 , E_1 and E_2 . Three different strains of type V mutants growing upon similar media. F_1 and F_2 . Mutant of type VIII growing upon similar media.

number of essentially similar strains were obtained to establish general cultural and morphological aspects, many other strains were observed which deviated from these patterns to a greater or lesser degree. In considering the cultural and morphological characteristics of various mutant types, attention will be given only to their most striking features. Detailed comparisons of cultures upon all media will not be reported.

To characterize successfully the various types of mutants it is necessary first to describe and illustrate the salient characteristics of the parent strain from which the mutants were derived.

The parent strain, culture NRRL 265, is entirely typical of the species *Aspergillus terreus* Thom and may be characterized as follows:

Colonies upon Czapek's solution agar growing rapidly at 25° to 30°C., attaining a diameter of 6 to 8 cm. in 10 to 14 days; 1 to 2 mm. deep; more or less irregular, with margin either dentate, lobed, or entire (fig. 1, A₁); plane, or developing radial furrows; zonate or azonate; uniform in color or with central area in somewhat lighter shades, heavy spor-ing throughout, with massed conidial heads lending colonies their distinctive color and texture; conidial heads arising largely from submerged hyphae and forming a dense stand, but also arising from raised tufts and scattered trailing vegetative hyphae in central areas; mature heads cinnamon to Sayal brown (Ridgway, Pl. XXIX)²; submerged mycelium colorless to light brown; exudate limited in amount, clear-amber in color; colony reverse in brown shades with the surrounding medium colored yellow. Conidial heads columnar, compact, of uniform diameter throughout (fig. 1, A₂) or with uppermost portion slightly expanded; commonly ranging from 35 to 55 μ in diameter, and from 150 to 500 μ or more in length depending upon age and other factors; light in color when young but ranging from cinnamon-buff through cinnamon to Sayal brown (Ridgway, Pl. XXIX) at maturity. Conidiophores somewhat sinuous, smooth-walled (fig. 1, A₃), colorless, usually arising from well-defined foot cells; ranging from 175 to 250 μ in length by 4.5 to 6.0 μ in diameter; wall comparatively heavy, ranging from 0.5 to 0.8 μ in thickness in upper portion to 0.6 to 1.0 μ in basal area. Vesicles domelike, with approximately the upper two-thirds bearing closely crowded sterigmata (fig. 1, A₃) ranging in diameter from 11 to 16.5 μ . Sterigmata in two series (fig. 1, A₃): primary sterigmata closely crowded, parallel, ranging from 5.0 to 5.6 μ in length by 1.6 to 2.0 μ in width, each bearing 2 or 3, rarely 4, secondary sterigmata; secondary sterigmata closely crowded, parallel, ranging from 5.8 to 6.6 μ by 1.2 to 1.8 μ . Conidia adherent in long chains, light golden in color when viewed under high magnifications; smooth-walled, slightly elliptical, 1.6 to 2.0 μ by 2.0 to 2.4 μ , or subglobose, 1.8 to 2.2 μ in diameter. Vegetative my-

celium hyaline, thin-walled, of variable diameter but generally 1.5 to 3.0 μ wide, irregularly and frequently branched, bearing occasional rounded to ovate bodies of 4.5 to 6.0 μ in diameter on short lateral branches.

On other commonly used substrata, such as malt and potato-dextrose agars, colonies grow more rapidly, are essentially plane, and are characterized by a more abundant growth of floccose aerial hyphae. Details of conidial structures and other morphological features are the same as those described above.

Type I. "Typical" albino.—This mutation occurred among the isolates produced by each of the three wave lengths employed. All strains duplicated one another and apparently differed from the parent culture only in the presence of unpigmented conidial heads. Mutants of this type are completely stable in culture. Strain 30-10-4 (ex. 2967 Å) illustrated in figure 1, B₁ to B₃ is representative. Yields of itaconic acid³ were approximately equal to those of the parent strain.

Type II. "Typical" buff.—This type was represented by a single isolate, strain 33-8-3 (ex. 2280 Å). It differs from the parent strain primarily in the color of its conidial heads which at maturity are light buff (Ridgway, Pl. XV) rather than cinnamon-brown. It appears to be somewhat intermediate between type I and the parent strain and is stable in culture. Acid production is comparable to the parent strain.

Type III. "Atypical" albino.—This type was represented by a single isolate, strain 30-9-12 (ex. 2967 Å). It produces colorless conidial heads of very atypical morphology. Heads are loosely columnar rather than compact and are comparatively short (fig. 1, C₂). Miniature droplets of clear exudate commonly collect on the heads and on drying lend to them irregular and commonly bizarre shapes. Sterigmata are normal in size, shape, and arrangement; but conidium formation is incomplete and abortive with the result that individual spores are not properly differentiated and adhere into long chains which fail to break up in liquid mounts (fig. 1, C₃). Septation in the chain is incomplete and individual conidia appear somewhat vacuolate. Viewed under low magnifications (dry) the spore mass seems to consist of colorless, slightly granular threads that commonly interweave with those of adjacent heads. The mutant is completely stable in culture. Acid production is very poor.

Type IV. Granulated.—Mutants of this type differ markedly from the parent strain in the appearance of the colony and in structural details. Colonies are light tan in color and upon Czapek's solution agar normally appear granulated from the irregular development of conidial heads (fig. 1, D₂). Heads

³ Data regarding itaconic acid production by mutants resulting from irradiated conidia have been generously supplied by Dr. Lewis B. Lockwood. See also Lockwood, Raper, Moyer, and Coghill. 1945. The Production and Characterization of Ultraviolet-Induced Mutations in *Aspergillus terreus*. III. Biochemical Characteristics of the Mutations.

² Ridgway, Robert. 1912. Color Standards and Color Nomenclature. 43 pp.; 53 colored plates. Washington, D. C.

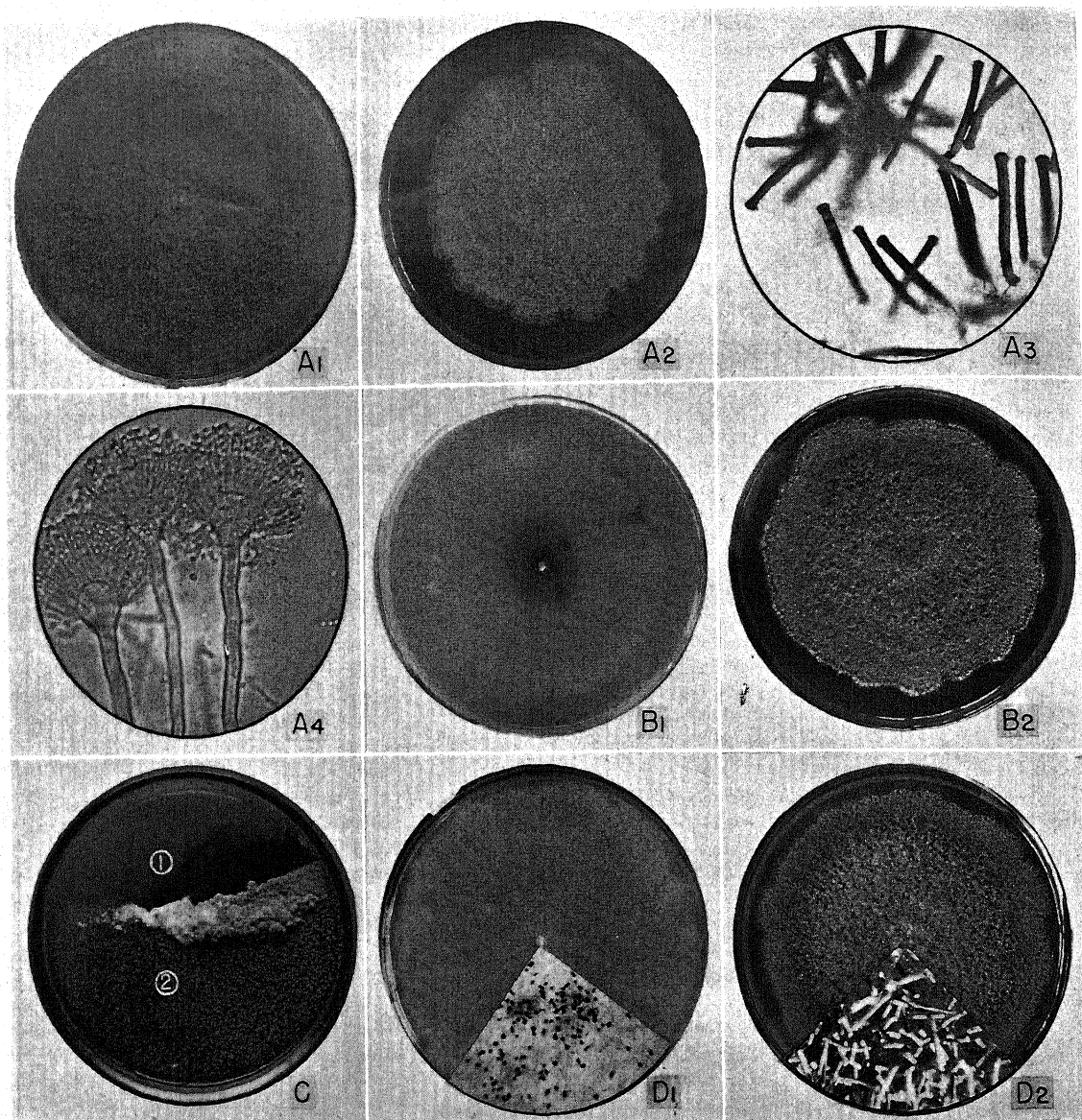


Fig. 3. Nutrient deficiency mutations in *Aspergillus terreus*, NRRL No. 265.—A. Strain 30-9-10 (type X) characterized by an inability to utilize nitrate nitrogen: A_1 , characteristic thin, spreading, light-sporing colony growing upon Czapek's solution agar with sodium nitrate. A_2 , heavy sporing, normal colony on malt extract agar; A_3 , typical columnar heads produced on Czapek's agar with sodium nitrate, $\times 25$; A_4 , photomicrograph showing normal character of same heads, $\times 450$.—B. Strain "Mutant C" (type IX) characterized by an inability to synthesize thiamin: B_1 , thin, nonsporulating colony on Czapek's solution agar; B_2 , wholly normal colony on malt extract agar.—C. Culture in which (1), mutant C and (2), strain 30-9-10 are planted opposite on a plate of Czapek's solution agar. Note heavy growth and spore production in frontier zone; no. 30-9-10 apparently produces thiamin for mutant C, whereas the latter reduces nitrate to ammonia which is utilized by strain 30-9-10.—D. Strain 30-8-1 characterized by an unknown nutrient deficiency: D_1 , colony growing upon Czapek's solution agar, thin, spreading and producing few and diminutive conidial heads (insert, $\times 10$); D_2 , normal, heavy sporing colony upon malt extract agar producing typical conidial heads (insert, $\times 10$).

are small, usually irregular in form, sometimes loosely columnar. Vesicles are normal in pattern and dimensions, but sterigmata are coarse and commonly do not show a normal separation into primary and secondary series (fig. 1, D_3). Mutants of this general type were isolated repeatedly. Strain 32A-S-

1-12 (*ex.* 2650 Å) as illustrated may be considered representative. It is comparatively stable in culture. Yields of acid are low.

Type V. White to yellow floccose.—This was the most abundant and most variable type encountered. No specific characteristics can be cited, since inter-

gradations obliterate any arbitrary limits that might be set up among different isolates. Generally speaking, however, colonies are floccose, often but not consistently restricted, white to yellow in color, light sporing, and grow about as well upon Czapek's solution as upon malt extract agar. Strain 30-9-5 (*ex* 2967 Å), illustrated in figure 1, E₁ to E₃, may be considered representative. As a rule, heads are not only limited in number but are commonly atypical in pattern; conidiphores may fail to develop vesicles, or vesicles, when formed, may fail to produce sterigmata and conidia (fig. 1, E₃). With few exceptions these forms are poor acid producers. Isolates showing different colony patterns, but falling within this general type, include those illustrated in figure 2 C, D, and E.

Type VI. Maroon exudate.—Colonies upon Czapek's solution agar are very restricted, orange-red to brown in color, and often produce an excessive amount of deep brown to maroon exudate (fig. 2, A₁). Conidial heads are produced sparingly and are small and somewhat atypical. Upon malt extract agar colonies duplicate the parent strain (fig. 2, A₂). The contrasting behavior upon these two media strongly suggests some nutritional deficiency as the basis of this mutation (see types IX and X). The type is reasonably stable in culture.

Type VII. Dark floccose.—To a degree this mutant represents the opposite of type VI. Colonies upon Czapek's solution agar are very rapidly growing (fig. 2, B₁), floccose, comparatively heavy sporing in marginal area after ten to fourteen days, and are characterized by the production of a rich brown to maroon coloration in the colony reverse. Conidial heads are of normal pattern and dimensions. Colonies upon malt extract agar, on the other hand, are restricted (fig. 2, B₂), with heads few in number and atypical in pattern. The mutant is stable in culture. It was isolated among mutants resulting from exposure to each of the three wave lengths. Strain 33-8-1 (*ex* 2280 Å) shown in figure 2, B₁ and B₂ is representative. Acid production is fair.

Type VIII. Leathery.—Colonies upon Czapek's solution agar are restricted, very close-textured, vary in color from buff to pale yellow-green, are strongly buckled and wrinkled, and produce few and atypical conidial structures. Colonies upon malt agar grow more rapidly, are plane, and comparatively thin, and bear few and atypical heads. Stable in culture. Strain 30-9-1 (*ex* 2280 Å), illustrated in figure 2, F₁ and F₂, is representative. Acid production varies with different strains.

Type IX. Thiamin deficient.—This mutant, commonly referred to as "mutant C" at the Northern Regional Research Laboratory, is represented by a single isolate (*ex* 2967 Å). The type is very distinctive: upon Czapek's solution agar colonies are thin, spreading, consisting almost exclusively of coarse, heavy-walled, submerged hyphae. A few atypical heads may be produced in the central area of the colony (fig. 3, B₁). On malt extract agar colonies duplicate the parent strain (fig. 3, B₂).

Dr. Lockwood¹ has shown that the peculiar growth of the mutant upon Czapek's solution agar results from a loss of the capacity to produce thiamin. When an adequate supply of this vitamin is incorporated into Czapek's solution agar, the mutant grows normally. The mutant is stable in culture and reinoculation of Czapek's medium from conidia produced in normal appearing cultures on malt agar reproduces the mutant characteristics. In the presence of adequate thiamin, yields of acid approximate those of the parent strain.

Type X. Nitrate.—Upon Czapek's solution agar, colonies are thin, rapidly spreading, consisting of a sparse, submerged vegetative mycelium giving rise to scattered but wholly typical conidial heads (fig. 3, A₁, A₃, and A₄). Colonies upon malt extract agar duplicate the parent strain in all essential characteristics (fig. 3, A₂). The mutant character of this type results from an inability of the mold to utilize nitrate nitrogen. Upon any substratum containing ammonia or amino nitrogen, the mutant appears normal (fig. 3, A₂). Mutants of this type were isolated repeatedly, and were obtained from spores subjected to each of the three wave lengths employed. They are completely stable in culture. Strain 32F-SS-1-1 (*ex* 2650 Å), illustrated in figure 3, A₁ to A₄, is representative. Acid production is variable: in some isolates approximating the parent, in others very poor.

Type XI.—This represents another deficiency mutant, but the deficiency in this case has not been identified. Colonies upon Czapek's solution agar are very thin with mycelium largely submerged. Unlike the preceding type, conidial heads produced on Czapek's agar are atypical in form and dimensions (fig. 3, D₁). Colonies upon malt extract agar are typical of the parent strain (fig. 3, D₂). The type is completely stable. Acid production is negligible.

HIGH ACID YIELDING MUTANTS.—In considering the different types of cultural mutants, notations have been made regarding their production of itaconic acid. It should be pointed out, however, that in the survey of cultures resulting from irradiated spores, Lockwood, Raper, Moyer, and Coghill (1945) found only 14 strains to produce acid in higher yields than the parent. These appear entirely normal in culture with two exceptions, which warrant special attention. On Czapek's solution agar strain 32D-6-3-3 (fig. 4, B₁) is characterized by close-textured, light tan colonies bearing comparatively few conidial heads that are somewhat reduced in size. On malt extract agar growth is more restricted and sporulation is even further reduced (fig. 4, B₂). This strain is of special interest since it produces itaconic acid of high purity (Lockwood, et al., 1945; Moyer and Coghill, 1945). In the case of most high-yielding strains, an appreciable proportion of the acid produced represents acids other than itaconic, and at times these may account for as much as 20 per cent of the acid present. In this strain, while total yields have not been

¹ Unpublished data.

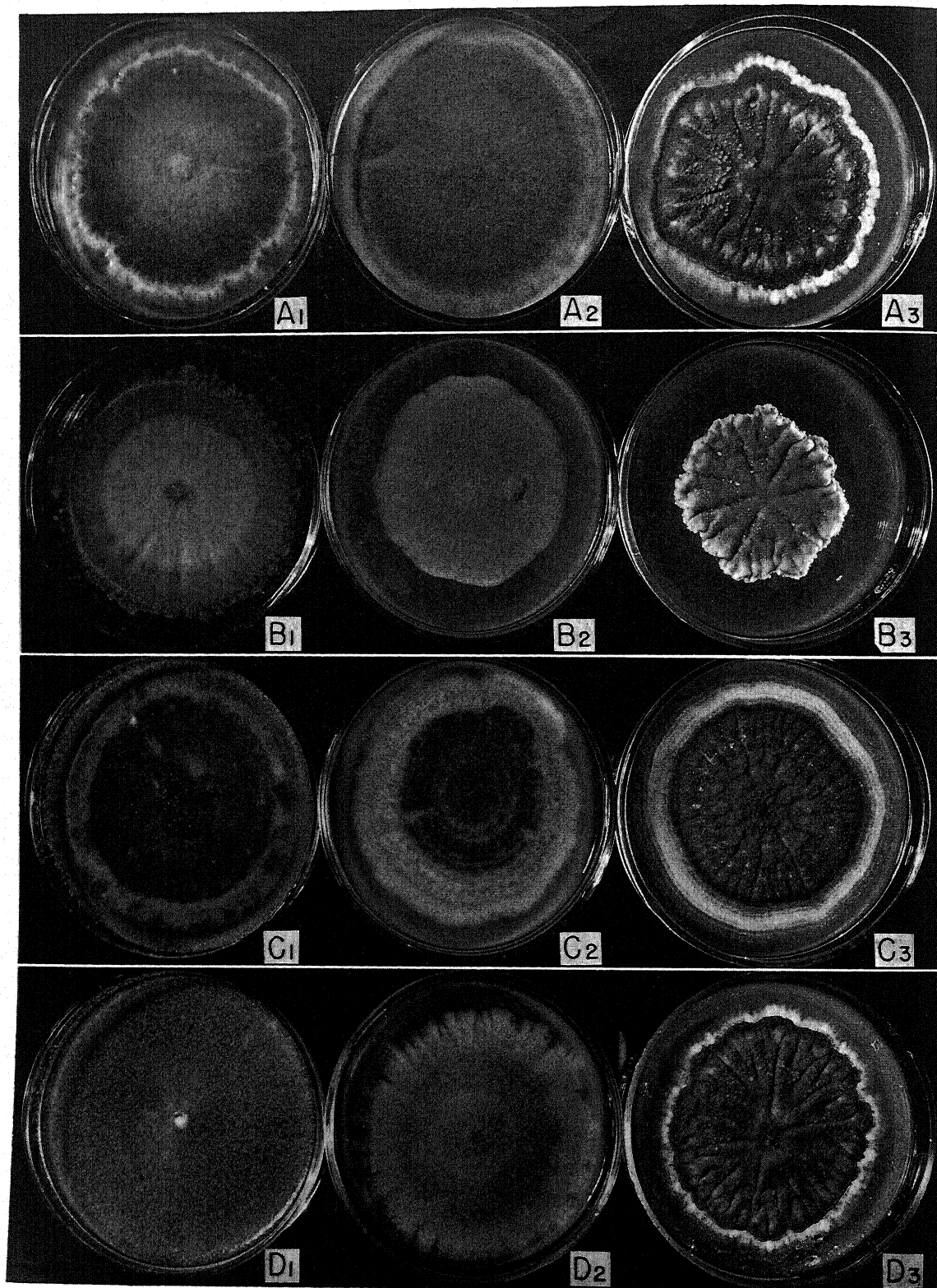


Fig. 4. Comparison of the parent strain and three mutations of *Aspergillus terreus*, NRRL No. 265, upon (1) Czapek's solution agar containing sodium nitrate, (2) malt extract agar, and (3) Czapek's solution agar containing ammonium nitrate. A_1 - A_3 , the parent strain upon the above media in the order listed. B_1 - B_3 , strain 32D-S-3-3, mutant char-

higher than those with the parent, itaconic acid has consistently represented almost 100 per cent of the total acidity.

The second exceptional strain, 32D-SS-4-1, is more nearly normal in character but differs from typical strains in producing colonies which spread more rapidly and grow more luxuriantly than the parent upon Czapek's solution agar (fig. 4, C_1). When grown upon Czapek's agar with NH_4NO_3 , it is essentially like the parent (fig. 4, C_3). This strain produces up to 20 per cent more acid than the parent culture, with the relative proportion of itaconic to other acids remaining approximately the same. The comparative growth of these two mutants, of the parent strain, and of the "nitrate" mutant (type X), upon standard Czapek's solution agar, Czapek's solution agar with NH_4NO_3 , and malt extract agar is shown in figure 4.

INFLUENCE OF THE CULTURE MEDIUM.—The great majority of strains, including normal appearing and cultural mutants alike, grew in approximately the same manner upon the standard Czapek's solution agar containing NaNO_3 and upon the medium in which NH_4NO_3 was substituted as a nitrogen source. Mutants of type X were exceptions. In general, the substitution of dextrose for sucrose had little effect, although in certain instances sporulation was somewhat greater upon the dextrose-containing medium. Substitution of urea for NaNO_3 yielded a medium upon which many forms produced more floccose colonies while others sporulated more abundantly. Type X grew normally upon this medium. Addition of 1 per cent corn steeping liquor, in general, tended to favor increased growth and sporulation, but altered the basic pattern of the colony only in types IX, X, and XI. Upon potato dextrose and malt extract agars strains generally appeared much alike, but with somewhat heavier growth and spore production on the latter. In a limited number of cases, e.g., type VII and certain strains belonging to type V, growth was substantially better upon Czapek's solution than upon malt extract agar. As a rule, however, more luxuriant growth and greater sporulation were obtained upon malt extract agar than upon any other medium employed. This response can be attributed to the presence of adequate sugars, nitrogen in the form of amino compounds, and necessary vitamins and growth factors.

By comparing the growth of the same cultures upon a synthetic medium such as Czapek's solution agar on the one hand and malt extract agar on the other, the investigator is at once struck by the effect of the substrate upon the types and numbers of mutants encountered. If, in the present study, the irradiated spores had been plated upon malt agar originally, and if subcultures in tubes had been

made upon this medium, at least five of the eleven mutant types recorded would not have become apparent. On the other hand, if malt extract agar had been used throughout this work, certain other types might have appeared which may have failed to grow upon Czapek's solution agar.

STABILITY OF MUTANTS.—Each of the isolates included in this comparative study was cultivated through ten successive culture generations upon Czapek's solution agar to determine whether or not mutations would prove stable. To make this test as conclusive as possible, the cultures were retransferred at intervals of from four to five weeks, thus allowing adequate time for light-sporulating strains to produce as many conidia as possible. New cultures were seeded from sporulating areas, never from hyphal tips or other purely vegetative structures. Incubation was at 30°C . In the great majority of cases the cultural picture produced by any given strain remained constant for each of the ten series. In some cases, however, cultures which at first appeared to represent mutations subsequently developed colonies wholly normal in appearance. In a few strains, there was a shift from mutant to apparently normal and back to mutant. In a very limited number of cases colonies resulting from irradiated spores which appeared normal at first subsequently appeared as cultural mutations. Variations of all types, however, were limited to a comparatively small number of strains, and by checking through the records of the different recultivations it was found that one strain shifted in appearance five times, four others changed four times, while still others shifted three times. It is of special interest that these "repeating" variations accounted for almost all of the isolates which shifted in appearance from one generation to another. It would thus appear that we can group the cultures resulting from irradiated spores along the following lines: (1) Mutants in which characters remain completely stable. This group, which would embrace more than 80 per cent of all isolates studied, would include certain physiological mutations as reflected in acid production, as well as cultural mutations of all but two or three of the eleven types cited above. (2) Mutants in which characters are unstable and may fluctuate within certain limits during periods of recultivation. This group is composed almost exclusively of isolates belonging to type V (which see).

DISCUSSION.—Working with species of *Neurospora*, Beadle and associates (Beadle and Tatum, 1941; Bonner, Tatum, and Beadle, 1943; Tatum and Beadle, 1942 and 1943) have succeeded in producing by X-ray treatment a whole series of deficiency mutations. Due to the destruction of specific genes, the ability to synthesize certain vitamins

acterized by the production of itaconic acid of high purity, upon the same media in like sequence. C_1 - C_3 , strain 32D-SS-4-1, mutant characterized by rapidly spreading colonies and high acid yields; media as in the preceding. D_1 - D_3 , strain 30-9-10 (type X), mutation characterized by an inability to utilize nitrate nitrogen; media as in the above. Note particularly the luxuriant growth of the latter upon Czapek's solution agar containing ammonium nitrate and how closely this colony duplicates that of the parent strain upon the same medium (A_3).

including thiamin, nicotinic acid, pantothenic acid, para-aminobenzoic acid, and pyridoxin was lost from certain strains. In other strains the ability to produce amino acids including methionine, lysine, arginine, leucine, isoleucine, tryptophane, and valine was similarly lost. When these necessary vitamins and amino acids were supplied, the mutant strains grew in an entirely normal manner.

In *Aspergillus terreus* we are probably dealing with the same type of phenomenon. In type IX, Dr. Lockwood has demonstrated that the mutant character which is so striking on Czapek's solution agar (fig. 3, B₁) reflects an inability of this strain to produce thiamin. Continued tests should further define this deficiency and establish whether it represents a thiamin deficiency *per se*, or an inability to produce thiazole or pyrimidine, or an inability to combine these two components of the thiamin molecule if separately supplied. In the case under consideration, the mutation may be assumed to result from a genetic change, and it becomes obvious as a particular nutritional and cultural response in the absence of adequate thiamin in the Czapek's solution agar. To the mycologist examining this culture, without knowledge of its underlying cause, it would appear as a mutant, variant, or saltant, depending upon the terminology selected, and would be so described. Not only is the cultural aspect of the mutant very bizarre, but individual fruiting structures, when produced, are atypical. In the presence of added thiamin, or upon natural substrata containing such, the culture grows and fruits in an entirely normal manner.

In type X it would seem probable that some gene, or gene combination, regulating nitrogen metabolism is involved. The parent strain grows luxuriantly upon media containing nitrate as a nitrogen source. The mutant, on the other hand, grows in a very atypical manner upon media containing sodium nitrate, potassium nitrate, or calcium nitrate as the sole nitrogen source but still produces normal fruiting structures. If nitrogen is supplied in the form of ammonium nitrogen, vegetative growth, as well as fruiting structures, is entirely typical. Thus the effect here is less far-reaching than that with the thiamin deficient type IX. Outwardly, only the rate and pattern of vegetative growth are changed. At the same time other changes more strikingly physiological in nature must occur independently of the nitrogen response, since among a number of apparently similar mutants some strains produced yields of itaconic acid approximately equal to the parent, while in other culturally similar strains the production of itaconic acid was quite low.

When strains mutant C (unable to produce thiamin) and 30-9-10 (unable to utilize nitrate nitrogen) are planted on opposite sides of a culture plate containing Czapek's solution agar, as shown in figure 3C, luxuriant growth is obtained in the frontier area when the mycelia of the two forms come into contact. Mutant C apparently reduces nitrate supplying ammonia ion for 30-9-10, the latter strain in turn produces thiamin necessary for optimum

growth and spore production by mutant C. Limited tests (still in progress) involving recultivation from selected conidial heads indicate that the two strains retain their distinctive characteristics in the common zone of greatly increased growth, but the possibility that heterokaryosis may occur has not been eliminated.

The abnormal growth and development of types VI and XI upon Czapek's solution agar and the normal behavior of these same types upon malt extract and other natural substrata indicate that these mutations also probably result from some type of nutritional deficiency. In neither case has the deficiency been identified. In a limited number of cases (for example, type VII) better growth was obtained upon Czapek's solution than upon malt extract agar (fig. 2, B). An explanation of this behavior is not obvious, since the latter medium undoubtedly represents the more complete nutrient of the two. The possibility of an altered metabolism, resulting in the production of an excess of staling products, or other substances inimical to growth and development of the fungus, should not be overlooked.

In addition to the mutations which appeared as definitely altered colonies upon one or more media, other types appeared which were more strictly morphological in character. Types I and II represent examples. In each case, growth was equally good upon Czapek's solution and malt extract agars, and in each case itaconic acid production remained at approximately the level of the parent strain. The only evident changes were (1) a complete loss of color in the conidial heads of type I, and (2) a substantial loss of color in the conidial heads of type II.

In types III and IV mutant characters were likewise constant irrespective of the substratum upon which the cultures were grown (see fig. 1, C and D), although they were more pronounced upon Czapek's solution agar and comparable synthetic media than upon malt extract agar and other natural substrata. Yields of acid were very low, indicating the presence of basic physiological changes as well as the more obvious morphological ones upon which the types were characterized.

Types V and VIII likewise represent basic morphological changes which are apparent upon all media tested. Variations in response upon the different media occurred, however, and maximum growth appeared in some cases upon Czapek's solution agar, and in other cases upon malt extract agar. Acid yields varied greatly among different strains, but in general they were quite low. It is apparent, therefore, that pronounced physiological changes accompanied morphological changes in these types also.

Mutations of varied types and degrees are represented among the isolates considered in the present paper. Some of these appear to represent comparatively simple mutations in which the capacity to produce some single essential substance, for example, thiamin in type IX, has been lost or in which the capacity to utilize some particular nutrient,

such as nitrate in type X, has been altered. In other cases physiological changes, as evidenced by an increased or decreased production of itaconic acid, have occurred which are not accompanied by any apparent cultural or morphological change. But in most cases physiological and morphological changes go hand in hand. In fact, it has become increasingly evident that the majority of isolates appearing as cultural or morphological mutations have their real bases in physiological changes, or altered biochemical reactions, as postulated by Tatum and Beadle (1943). To mycologists, and particularly those working in taxonomy, this relationship poses a real problem. If induced mutations, which appear culturally distinct, generally result from altered biochemical reactions, how then are we to interpret culturally different forms as they are isolated from nature? Certainly, newly isolated forms should not be described as new species or varieties until they have been carefully compared with known and stable species and varieties upon different substrata. On the other hand, one would not seem justified in designating such distinctive forms from nature as mutations when their parentage cannot be positively established.

Strains of *Aspergillus terreus* that are newly isolated from natural sources show appreciable variation when large numbers of isolates are compared. Differences occur in the general character and coloration of colonies, in the amount of sporulation, and to some degree in the form and pattern of conidial heads. Such variation, however, is not so great as that encountered among the mutations produced by ultraviolet radiation from the single strain NRRL 265. Whereas variant types in the two cases bear some resemblance, they can in no sense be said to duplicate one another. Strains characterized by yellow-floccose colonies, which were most abundant among the ultraviolet-induced mutations, are very rarely isolated from nature, and strains with white heads have not been reported for this species. Yuill (1939), however, has isolated naturally occurring mutants of *Aspergillus nidulans* and *Aspergillus fumigatus* characterized by white and very pale buff heads, respectively, and it appears reasonable that such types should exist for *A. terreus*.

Increased production of itaconic acid by strains resulting from irradiated spores was limited to a comparatively small percentage of the strains tested (p. 171), and in no case was the increased yield spectacular. Repeated tests, however, demonstrated some increase from a limited number of isolates, in some cases amounting to approximately 20 per cent. Other mutations which may conceivably prove important are those yielding itaconic acid of high purity, even though total yields may not exceed those of the parent. Based upon the present studies and the work of Lockwood, Raper, Moyer, and Coghill (1945) and Moyer and Coghill (1945), the production of ultraviolet-induced mutations appears to offer definite, if somewhat limited, possibilities of increasing yields of itaconic acid from *A. terreus*. In terms of prevailing analyses of induced muta-

tions, it is realized that only a comparatively small number of strains have been surveyed. The cultural operations and involved chemical analyses incident to the proper conduct of such a survey, however, have precluded the testing of a larger number of isolates.

During the period that the isolates from irradiated spores were being tested, isolations of *A. terreus* were made from soils and other natural sources. More than 300 such isolates have now been tested and among them a limited number of strains have been found which produce substantially higher yields of itaconic acid than any of the mutants resulting from irradiated conidia of NRRL 265. Having shown that yields in a given strain (NRRL 265) can be increased, it is now our hope similarly to subject conidia of one or more of the more recently isolated and better-producing strains to ultraviolet radiation.

SUMMARY

Conidia of a selected strain of *Aspergillus terreus* were exposed to ultraviolet radiation, and random isolations were made from colonies resulting from irradiated cells. Approximately two hundred of these were cultivated upon a variety of agar media to determine the effect of the substratum upon growth and attendant cultural characteristics. The majority of isolates from irradiated spores, normal appearing and cultural mutants alike, remained stable when recultivated through ten successive culture generations upon Czapek's solution agar. Eleven types of mutations were recognized. One of these resulted from an inability of the mutant to produce thiamin. Another was unable to utilize nitrate nitrogen but developed normally if supplied with ammonia or amino nitrogen. In other cases mutations were more strictly morphological and consisted of a total or partial loss of color from the conidial heads without apparent alteration in physiology. In still other and more numerous cases, both morphological and physiological changes were observed. Cultural and morphological differences commonly reflected basic physiological or biochemical changes. Of more than 200 isolates resulting from irradiated spores tested for the production of itaconic acid, only 14 produced yields greater than the parent strain. Among this number only two appeared as cultural mutants, and only one of these was markedly different from the parent strain. The production of ultraviolet-induced mutations appears to offer definite, if somewhat limited, possibilities of increasing yields of itaconic acid from *Aspergillus terreus*.

FERMENTATION DIVISION,
NORTHERN REGIONAL RESEARCH LABORATORY,
BUREAU OF AGRICULTURAL AND INDUSTRIAL
CHEMISTRY,
AGRICULTURAL RESEARCH ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE,
PEORIA, ILLINOIS
INDUSTRIAL HYGIENE RESEARCH LABORATORY,
NATIONAL INSTITUTE OF HEALTH,
BETHESDA, MARYLAND

LITERATURE CITED

- BEADLE, G. W., AND E. L. TATUM. 1941. Genetic control of biochemical reactions in *Neurospora*. Proc. National Acad. Sci. 27: 499-506.
- BONNER, D., E. L. TATUM, AND G. W. BEADLE. 1943. The genetic control of biochemical reactions in *Neurospora*: A mutant strain requiring isoleucine and valine. Archives of Biochem. 3: 71-91.
- CALAM, C. T., A. E. OXFORD, AND H. RAISTRICK. 1939. The biochemistry of micro-organisms. LXIII. Itaconic acid, a metabolic product of a strain of *Aspergillus terreus* Thom. Biochem. Jour. 33: 1488-95.
- EMMONS, C. W., AND ALEXANDER HOLLAENDER. 1939. The action of ultraviolet radiation on dermatophytes. II. Mutations induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. Amer. Jour. Bot. 26: 567-75.
- HOLLAENDER, ALEXANDER, AND WALTER D. CLAUS. 1936. The bactericidal effect of ultraviolet radiation on *Escherichia coli* in liquid suspensions. Jour. Gen. Physiol. 19: 753-65.
- , AND B. M. DUGGAR. 1938. The effects of sublethal doses of monochromatic ultraviolet radiation on the growth properties of bacteria. Jour. Bact. 36: 17-37.
- , KENNETH B. RAPER, AND ROBERT D. COGHILL. 1945. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. I. Production of the mutations. Amer. Jour. Bot. 32: 160-165.
- LOCKWOOD, L. B., KENNETH B. RAPER, A. J. MOYER, AND R. D. COGHILL. 1945. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. III. Biochemical characteristics of the mutations. Amer. Jour. Bot. (In press.)
- MOYER, A. J., AND R. D. COGHILL. 1945. The laboratory-scale production of itaconic acid by *Aspergillus terreus*. Jour. Amer. Chem. Soc. (In press.)
- TATUM, E. L., AND G. W. BEADLE. 1942. Genetic control of biochemical reactions in *Neurospora*: An amino-benzoicless mutant. Proc. Nat. Acad. Sci. 28: 234-43.
- . 1943. The relation of genetics to growth factors and hormones. 4th Growth Symposium 27-35.
- THOM, CHARLES. 1930. The Penicillia. 644 pp. The Williams and Wilkins Co. Baltimore.
- YUILL, EDWARD. 1939. Two new *Aspergillus* mutants. Jour. of Bot. (London). pp. 174-175, Pl. 618.

STUDIES ON COLCHICINE-INDUCED AUTOTETRAPLOID BARLEY.

III. PHYSIOLOGICAL STUDIES¹

Shao-Lin Chen and P. S. Tang

THE MORPHOLOGICAL and cytological characteristics of some colchicine-induced autotetraploid barley plants have been given in the previous paper of this series. The present account reports some physiological characteristics of these tetraploid plants.

RESPIRATION.—Oxygen consumption and carbon dioxide production by the germinating seeds of 2n and 4n barley were measured by means of the microrespirometers of Warburg as used by one of us in previous measurements of respiration in germinating seeds (Tang, 1931). Seven dehulled seeds which had been germinating at 24°C. for 36 hours were placed in the main portion of the respirometer vessel. The inset contained 0.2 ml. 10 per cent NaOH when oxygen consumption was to be measured, and water when carbon dioxide production was to be measured. Two respirometers were run at a time beside the thermobarometer, one for the 2n seeds and the other for the 4n seeds. The experiments were performed at 18°, 24° and 28°C. At the end of the experiments the seeds were ground and dried to constant weight and their nitrogen contents were estimated by micro-Kjeldahl.

Table 1 gives the rates of O₂-consumption and CO₂-production of the germinating seeds of the 2n and 4n barley plants used. It is seen from this table that both the rates of O₂-consumption and CO₂-production of the 4n seeds were lower than those of the 2n seeds, whether expressed in terms of dry-weight or of nitrogen content. Taken as a whole, the respiratory quotients of the 4n seeds were found to be lower than those of the 2n seeds.

¹ Received for publication September 14, 1944.

Manuscript received through the Department of State, Washington, D. C., and published under a special ruling of the Editorial Board.

TABLE 1. O₂-consumption and CO₂-production of germinating barley seeds at different temperature (seven seeds used in each experiment).

Temp. °C.	Q _{O₂}	Q _{CO₂}	R.Q.	cmm. O ₂ mg. N	cmm. O ₂ g. dry wt.	cmm. CO ₂ mg. N	cmm. CO ₂ g. dry wt.	Q ₁₀ for O ₂
Diploid barley								
18°	58	47	.81	18.8	378.1	15.2	306.6	..
24°	105	96	.91	33.9	687.8	30.8	621.2	3.4
28°	199	184	.92	64.5	1294.7	59.4	1195.9	..
Dry weight = 0.154 gm. (for 7 seeds).								
Total nitrogen = 3.1 mg. (for 7 seeds).								
Autotetraploid barley								
18°	105	81	.76	17.3	365.4	13.3	281.1	..
24°	189	157	.83	30.8	655.7	25.8	545.5	2.8
28°	301	274	.91	49.5	1044.0	45.1	951.6	..
Dry weight = 0.288 gm. (for 7 seeds).								
Total nitrogen = 6.07 mg. (for 7 seeds).								

The R.Q. values for both kinds of seeds, however, changed with temperature, agreeing with a previous observation to this effect in *Lupinus albus* (Tang, 1932). The values of the temperature quotients for O₂-consumption were different in the two cases, being 3.4 for the 2n seeds and 2.8 for the 4n seeds.

That the lower rate of respiration in the 4n seeds is an inherent characteristic of the cells, and not due to the fact that they may contain less living matter in proportion to the reserve materials is shown by the following experiment. The embryo and endosperm were separated from each other in one hundred 2n and one hundred 4n seeds. The two portions were dried and weighed separately. No difference was found in the ratio of weight of embryo to weight of endosperm in the two types of seeds.

TRANSPIRATION.—The rates of transpiration of 55-day-old 2n and 4n barley plants were measured by means of simple potometers.

Seven 2n and an equal number of 4n plants, all at the 4-leaf stage, were used, each placed in a separate potometer. The experiment lasted from noon to 10 P.M., at the end of which the leaf area and dry weight of the seedlings were ascertained.

The average amount of water transpired by the seven 2n seedlings during the 10 hours of exposure was 0.38 ml. per square centimeter of leaf area. That for the seven 4n seedlings was 0.26 ml. per square centimeter. This difference becomes more apparent when the rate is expressed in terms of ml. transpired per unit dry-weight. In that case, the respective values become 78.3 ml. per gm. and 38.8 ml. per gm.

OSMOTIC CONCENTRATION AND SUCTION PRESSURE.

—The method used in the estimation of osmotic concentration and suction pressure of the cells of the 2n and 4n barley plants was that of Ursprung as modified by Molz (Barton-Wright, 1937). Strips of young leaves were removed from the plants and were measured under the microscope, first in paraffin oil, and then in various concentrations of sucrose solution. The change in length of the strips in these solutions was plotted against the concentration of sucrose solution, as shown in figure 1. At the sucrose concentration where the curves cut the zero axis, there was no change in the lengths of the strips, and those points of interception represent the suction pressures of the cells in the leaves of the 2n and 4n plants.

In figure 1, these values correspond to 0.75 M sucrose solution for the 2n plants, and 0.875 M

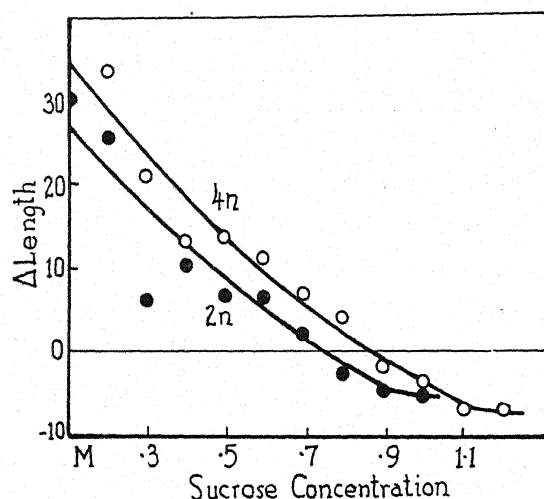


Fig. 1. Determination of osmotic concentration and suction pressure in autotetraploid and diploid barley. Open circle for 4n barley, closed circle for 2n. Δ length refers to change in length of the strips in arbitrary units as read from ocular micrometer.

sucrose solution for the 4n plants. At the point of the curves in figure 1 where the lengths of the strips failed to undergo further reduction with increase in sucrose concentration, and where plasmolysis just began, the concentration of the external sucrose solution was equivalent to the maximum osmotic value of the cell sap. This concentration was found to be 0.9 M for the 2n plants and 1.1 M for the 4n plants.

IMBIBITION.—Imbibition of water by the seeds of 2n and 4n plants was studied simply by placing the seeds on moist filter paper in petri dishes and weighing them at 3-hour intervals. The increase in weight, expressed on the basis of initial dry-weight of the seeds, gave the amount of water imbibed. Twenty dehulled seeds each, from the 2n and 4n lots, were used in these experiments, and the results are given in table 2. Up to the thirtieth hour, at the time when the seeds were to germinate, the 4n seeds took up 0.77 gm. of water per gm. initial dry-weight while the 2n seeds took up only 0.6 gm., indicating a higher imbibition capacity in the 4n seeds.

CHEMICAL COMPOSITION.—The following analyses were made on the 2n and 4n barley seeds. Protein content was estimated with micro-Kjeldahl, lipid substances with ether extraction in Soxhlet, ash by ignition, and other nitrogen-free compounds, presumably carbohydrates, by difference. The results of the analyses are given in table 3.

It is seen from this table that the 2n seeds contained a little more water and carbohydrates, while the 4n seeds contained more protein nitrogen, lipid substance and ash.

The results of the experiments indicate that certain distinct differences exist between normal diploid barley plants and those autotetraploid plants induced by colchicine. One of these concerns the rate of metabolism, the rate of respiration in the tetraploids being lower, and this was shown to be an inherent characteristic of the tetraploid cells,

TABLE 2. Imbibition of water by barley seeds.

Time (in hrs.)	Wt. of 4n seeds (in gm.)	Gram of water imbibed	Wt. of 2n seeds (in gm.)	Gram of water imbibed	gm. H ₂ O imbibed by 4n seeds	gm. H ₂ O imbibed by 2n seeds
					gm. initial dry weight	gm. initial dry weight
0	.869809
3	1.104	.235	.950	.141	.27	.17
6	1.220	.351	1.039	.230	.40	.28
9	1.283	.414	1.083	.274	.48	.34
12	1.330	.461	1.127	.318	.53	.39
15	1.376	.507	1.159	.350	.58	.43
18
21	1.433	.564	1.206	.397	.65	.49
24	1.459	.590	1.231	.422	.68	.52
27	1.483	.614	1.254	.455	.71	.55
30	1.541 ^a	.673	1.298 ^a	.489	.77	.60
33	1.567	.698	1.318	.509	.80	.63

^a Already germinated.

TABLE 3. Chemical composition of 2n and 4n barley seeds.

	2n barley	4n barley
Water content ^a	5.7%	5.3%
Protein	12.7%	14.7%
Ether extract	2.2%	2.6%
Ash	1.6%	2.5%
Other N-free compounds.....	83.5%	80.2%

^a Water content is calculated on air-dried weight basis while other determinations are on oven-dried weight bases.

and not merely a result of the relative proportion of living and reserve matter in the seeds. This lowered rate of metabolism may bear a relationship to the slower rate of growth and development in the tetraploids. It cannot be said as yet, however, whether the former is the cause of the latter, or *vice versa*.

The transpiration rate in the 2n plants was higher than that of the 4n plants by 50 per cent, when expressed in terms of leaf-area, and 100 per cent when expressed in terms of dry-weight. This indicates a higher water economy, or transpiration efficiency in the 4n plants. This, coupled with the higher osmotic pressure and suction pressure in the cells of the tetraploids may account for the fact that during the course of these experiments, the tetraploids were found to be able to withstand drying better than the normal diploids. In general, polyploids are known to be more hardy and more adaptable to adverse environmental conditions than diploids (Müntzing, 1936; Stebbins, 1940).

The nitrogen and ether-extractable matter, as well as ash, in our tetraploid barley were found to

be higher than those in the seeds of the normal diploid plants. This is in accord with many observations where changes in chemical composition of the plants occur as a result of chromosome multiplication. Among these may be mentioned the increase in vitamin C content (Sansome and Zilva, 1933) and protein nitrogen (Kostoff and Axamitnaja, 1935) in tetraploid tomato; the increase in nicotine content in tetraploid tobacco (Noguti et al., 1940), and increase in sugar content in triploid sugar beet (Peto and Boyes, 1940).

SUMMARY

The rates of oxygen consumption and of carbon dioxide production by germinating 4n barley seeds were found to be lower than those of the normal diploids. Tetraploid barley seedlings transpired at a lower rate than the diploids whether expressed on a leaf-area or on a dry-weight basis. Tetraploid barley cells possessed a suction pressure corresponding to 0.875 M sucrose solution, and a maximum osmotic pressure of 1.1 M sucrose solution, while the corresponding figures for the diploids were 0.75 M and 0.9 M respectively. Placed on moist filter paper, the seeds of 4n barley imbibed more water per unit dry-weight than did those of the diploids. The seeds of the 4n barley plants contained more protein nitrogen, lipid substance and ash than those of the diploids. On the other hand, the latter contained more N-free matter, presumably carbohydrates, than the former.

PHYSIOLOGICAL LABORATORY,
TSING HUA UNIVERSITY,
KUNMING, CHINA

LITERATURE CITED

- BARTON-WRIGHT, E. C. 1937. General plant physiology. Williams and Norgate, London.
- KOSTOFF, D., AND I. A. AXAMITNAJA. 1935. Studies on polyploid plants. VII. Chemical analysis of F_1 -hybrids and their amphidiploids. *Compt. Rend. Acad. Sci. U.S.S.R.* 1:325-329.
- MÜNTZING, A. 1936. The evolutionary significance of autopolyploidy. *Hereditas* 21:263-378.
- NOGUTI, Y., H. OKA, AND T. OTUKA. 1940. Studies on polyploidy in *Nicotiana* induced by the treatment with colchicine. II. Growth rate and chemical analysis of diploid and its autotetraploid in *Nicotiana rustica* and *N. tabaccum*. *Jap. Jour. Bot.* 10:343-364.
- PETO, F. H., AND J. W. BOYES. 1940. Comparison of diploid and triploid sugar beet. *Can. Jour. Res.* 18: 273-282.
- SANSOME, F. W., AND S. S. ZILVA. 1933. Polyploidy and vitamin C. *Biochem. Jour.* 27:1935-1941.
- STEBBINS, G. L., JR. 1940. The significance of polyploidy in plant evolution. *Amer. Nat.* 74:54-66.
- TANG, P. S. 1931. Temperature characteristics for the oxygen consumption of germinating seeds of *Lupinus albus* and *Zea Mays*. *Jour. Gen. Physiol.* 14:631-641.
- . 1932. On the respiratory quotient of *Lupinus albus* as a function of temperature. *Jour. Gen. Physiol.* 15:561-569.

STUDIES ON COLCHICINE-INDUCED AUTOTETRAPLOID BARLEY.

IV. ENZYME ACTIVITIES¹

Shao-Lin Chen and P. S. Tang

IN THE previous papers of this series (Chen, Shen, and Tang, 1945; Chen and Tang, 1945) certain morphological and physiological characteristics of the 2n and 4n barley plants and seeds were compared. This account deals with the enzyme activities of the seeds and seedlings of the 2n and 4n barley.

CATALASE ACTIVITY.—About 2 mg. of finely ground powder of barley seeds, after being dried in a desiccator, were transferred into the main portion of a Warburg microrespirometer vessel, to which 1 ml. of distilled water was added. Following this, 0.5 ml. of 3 per cent hydrogen peroxide was pipetted into the side-arm of the vessel. The vessels were placed in a thermostat at 20°C. for 30 minutes after which the tap was closed, the contents in the side-arm mixed with the powder in the main vessel, and readings were taken at 5-minute intervals at 20°C. until no further evolution of O₂ was observed.

The difference between autotetraploid and diploid barley was rather striking. The former yielded 58 cmm. of O₂, while the latter evolved only 23 cmm. That is, the seed powder of autotetraploid barley showed about 2.5 times the catalase activity of the diploids.

The time course of catalase reaction was found to resemble that for a first order chemical reaction, i.e.

$$\frac{dx}{dt} = k(a - x).$$

In this case, however, the substrate concentrations *a* and *x* were not directly measured. If the assumption is made that the amount of O₂ evolved is proportional to substrate concentration (see Tang, 1941), then *a* corresponds to the total amount of oxygen evolved (*A*) during the course of the reaction; *x* corresponds to *y*, the amount of oxygen evolved at any time *t*, and the rate of the reaction, as given by *Q*, is a first order function of *A*—*y*:

$$Q = f \frac{dx}{dt} = k'(a - x) = k'(A - y). \quad (1)$$

The data for the catalase activities of the 2n and 4n barley seeds used in the experiment are plotted in figure 1 according to equation (1) and it is seen that for the most part the course of the decomposition of H₂O₂ followed the first order chemical reaction, except for the few points towards the end of the reaction which deviated from the straight line as demanded by the equation. From figure 1, the mean constants obtained for the two cases were:

¹ Received for publication September 14, 1944.

Manuscript received through the Department of State, Washington, D. C., and published under a special ruling of the Editorial Board.

$k' = 3.23 \times 10^{-2}$ for the 2n, $k' = 2.73 \times 10^{-2}$ for the 4n barley seeds.

MALT DIASTASE ACTIVITY.—Barley seeds were allowed to germinate at room temperature and were air-dried when the radicles reached 2–3 cm. in length. Malt powder for experimentation was obtained by grinding these germinated seeds into fine powder.

The diastatic value of malt extract was ascertained by the method of Lintner (Ross-Mackenzie, 1927); 0.8 gm. of malt powder was mixed with one liter of distilled water and kept at room temperature for 6 hours. Into a series of 10 test tubes were delivered 1, 2, 3, . . . to 10 ml. of the filtered extract, and to each of them 5 ml. of 2 per cent starch solution was added. Each tube was then made up to 15 ml. with distilled water. The tubes were well shaken and kept at 20°C. for an hour, at the end of which 1 ml. of Fehling's solution was added to each of them. The tubes were then placed in a boiling water bath for 20 minutes. The tube containing the quantity of enzyme which was just sufficient to reduce the added copper in that period, as shown by the complete disappearance of blue color, was used to calculate the standard value of the enzyme solution.

In our experiment, the fifth tube in the 4n barley series, and the ninth tube in the 2n series produced just enough sugar to reduce completely 1 ml. of Fehling's solution. If we assign the value of 100 to a solution containing 1 ml. of malt extract and producing in 1 hour enough sugar under the outlined conditions, then the diastatic power in our case may be calculated by the following formula:

$$D = \frac{100}{V},$$

where *D* = diastatic activity of the enzyme preparation and *V* = the minimum volume of malt extract which produced enough sugar in one hour to reduce 1 ml. of Fehling's solution. Thus the relative diastatic activity of the 4n malt extract was 20, and that of the 2n malt extract was 11. In other words, the diastatic activity of the sprouted 4n barley seeds used in this experiment was approximately twice that of the diploids.

DEHYDROGENASE ACTIVITY.—Various kinds of dehydrogenase activity in the 2n and 4n barley seedlings were studied with the methylene blue technique of Thunberg (1917) with the following modifications (see Chen and Tang, 1941). Equal amounts of fresh leaves (about 1 gm.) of the two kinds of barley seedlings were ground and extracted with 6.8 phosphate buffer. Three ml. of the buffer extract was transferred into a small vial to which 0.5 ml. of various kinds of substrates were added. After the addition of 0.2 ml. of a 0.05 per cent

methylene blue solution, the liquid surface in each vial was covered with a layer of liquid paraffin to obtain an anaerobic condition. Time required to reduce completely the methylene blue to its leucoform was recorded as a measure of dehydrogenase activity. The results of the experiment are tabulated in table 1.

TABLE 1. Rate of reduction (in minutes) of methylene blue by 2n and 4n barley leaf extracts.

Substrate	4n barley	2n barley
Succinic acid (0.1 M).....
<i>l</i> -malic acid (0.1 M).....
Lactic acid (0.1 M).....
Citric acid (0.1 M).....
Ca-glycerophosphate (0.05 M).....
Ethyl alcohol (0.1 M).....	170	140
Glycine (0.1 M).....	380	170
<i>dl</i> -alanine (0.1 M).....	140	105
Glutamic acid (0.1 M).....	320	105
Cysteine (0.1 M).....	230	80
<i>l</i> -cystine (0.001 M).....	320	200
Control.....

From table 1 it may be concluded that in both the 2n and 4n barley seedlings the nature of the dehydrogenase was the same, since they were both without effect on succinic acid, *l*-malic acid, lactic acid, citric acid, and Ca-glycerophosphate. They both effected the reduction of methylene blue in the presence of ethyl alcohol, glycine, *dl*-alanine, glutamic acid, cysteine, and *l*-cystine. The rates at which these compounds were oxidized, however, differed between the 2n and 4n plants. In general, the 2n barley extract was more active than that of the 4n plants, and in the case of cysteine and glutamic acid, the ratio of the speed of reduction amounted to about 3:1. This difference in dehydrogenase activity between the two kinds of barley plants appears to agree with the results obtained in respiration experiments where the 2n plants respired faster than the 4n plants (Chen and Tang, 1945).

SUMMARY

The catalase activities of the 2n and 4n barley seeds followed a first order reaction curve, the constants of the reaction being $k' = 3.23 \times 10^{-2}$ for 2n seeds, and $k' = 2.75 \times 10^{-2}$ for the 4n seeds. The rate of decomposition of H_2O_2 by the 4n seeds was 2.5 times faster than that of the 2n seeds.

The malt diastase activity of sprouted seeds of 4n barley was about twice that of the 2n seeds as measured by the amount of extract necessary to reduce a given amount of Fehling's solution in a given time.

Extracts from the leaves of 2n and 4n seedlings were both without effect on succinic acid, *l*-malic acid, lactic acid, citric acid, and Ca-glycerophos-

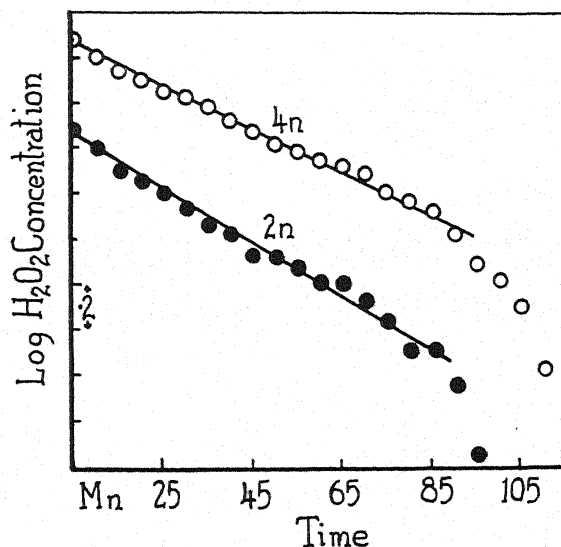


Fig. 1. Log concentration of H_2O_2 as expressed in terms of amount of oxygen evolved plotted against time. Open circles for 4n and closed circles for 2n barley seeds.

phate, but they all effected a reduction of methylene blue in the presence of ethyl alcohol, glycine, *dl*-alanine, glutamic acid, cysteine and *l*-cystine. The rate of reduction was in general faster in the presence of 2n extracts than 4n extracts.

PHYSIOLOGICAL LABORATORY,
TSING HUA UNIVERSITY,
KUNMING, CHINA

LITERATURE CITED

- CHEN, H. K., AND P. S. TANG. 1941. A simplified Thunberg technic for metabolic studies. Sci. Report, Tsing Hua Univ. Ser. B. 3:8.
- CHEN, S. L., S. M. SHEN, AND P. S. TANG. 1945. Studies on colchicine-induced autotetraploid barley. I and II. Cytological and morphological observations. Amer. Jour. Bot. 32:103-106.
- CHEN, S. L., AND P. S. TANG. 1945. Studies on colchicine-induced autotetraploid barley. III. Physiological studies. Amer. Jour. Bot. 32:177-179.
- ROSS-MACKENZIE, J. 1927. Brewing and malting. The Technical Press, London.
- TANG, P. S. 1941. Respiration in the living cell. Quart. Rev. Biol. 16:173-189.
- THUNBERG, T. 1917. Zur Kenntnis der Einwirkung tierischer Gewebe auf Methylenblau. Skand. Arch. Physiol. 35:163.

GROWTH AND PHOSPHORUS ACCUMULATION IN COTTON FLOWERS AS AFFECTED BY MEIOSIS AND FERTILIZATION¹

Orlin Biddulph and Donald H. Brown

THE OBJECT of this investigation was to "mark" an amount of phosphorus with a radioactive marker,² place this in a nutrient solution and allow a given plant to absorb from it a definite amount of the phosphate. Two days were then allowed while the phosphate was thoroughly circularized throughout the plant and delivered in characteristic quantities to the many flowers and fruits on the plant. The phosphate which entered each flower or fruit during such a two-day period could be distinguished by its radioactivity from that which was previously present, and furnished a detailed record of the current trends of phosphorus intake by flowers or fruits in various stages of development. The cotton plant was chosen as experimental material because it produces numerous large flowers in a continuous series so that at a given time one plant may possess fifty to seventy buds or fruits representing all stages of development. Specific attention has been given to the relationship between the morphological events of microsporogenesis, megasporogenesis, and fertilization and the accumulation of dry weight, water, and phosphorus. The records obtained from five individual plants furnished the basis for this investigation.

Much has been written about the water and mineral balance within the plant as a whole during the inception of flowering and fruit formation (Loehwing, 1942) but no complete history of the mineral uptake by the developing flowers as influenced by the various morphological events associated with reproduction seems to exist. Such a history is presented here for phosphorus and in so doing, a similar study for dry weight increase, and consequently water balance, was necessary.

METHODS.—The plants were grown from seed obtained from the Mississippi State Agricultural College. The nutrient solution was an aerated Shive-Robbins solution (1937), contained in 160-liter tanks. Fluorescent lights supplemented the rather dull days of the winter months. The temperature was thermostatically controlled at 21°C. (minimum). The maximum temperature during the winter was only a few degrees above this, but it rose considerably on sunny days in the spring.

The plants reached the proper stage of flowering at the age of 5½ months (by the latter part of March, 1942). At this time five were selected for study. Each one, in succession, was carried through the following treatment: (1) All flowers and fruits were measured as to length of parts and as to position on the plant (distance from base of stem to branch, and distance along branch to flower). (2)

The plant was then transferred from the large tank to a 6-liter enameled refrigerator pan containing a similar aerated nutrient solution, except that the phosphorus in the solution was "marked" by the addition of the radioactive isotope ^{32}P . The plant remained in this solution for 48 hours, after which it was transferred to a similar pan of ordinary nutrient solution (no radiophosphorus) for 48 hours more. (3) At the close of this period the flowers and fruits were again measured as to length of parts. From those flowers which were near microsporogenesis a few anthers were removed, fixed in Farmer's solution and later examined by the acetocarmine smear technique to determine the stage of microsporogenesis. (4) After measurement each flower or fruit was removed, weighed to the nearest 5 milligrams, then dried and weighed to the nearest milligram, and finally ground and an aliquot weighed for analysis. The material was then ashed at dull red heat and the amount of "marked" phosphate taken up during the experimental period was determined with a Geiger-Muller counter (Dunning and Skinner, 1935) and recording circuit (Evans and Alder, 1939). Each flower on the fifth plant was analyzed for total phosphorus (radioactive and non-radioactive together) by the procedure of Zinzadze (1935).

The six liters of nutrient solution, after the radiophosphorus was added, contained 0.937 grams of phosphorus, of which 1090 micro-curies were radioactive. This is a ratio of 0.860 milligrams of phosphorus per micro-curie of radioactive phosphorus on March 21, 1942. The phosphorus was calculated to PO_4 and designated as P^*O_4 to indicate that it was "marked" with the radioactive isotope.

In using radiophosphorus for experimental work, and in the concentrations herein employed, two assumptions seem justifiable, i.e., (1) that the percentage difference in the atomic weights of the two atoms, P^{31} and P^{32} is not great enough to cause differences in the rate of absorption and translocation of the phosphate which exceed the experimental error of the determinations; and (2) that the amount of ionization occurring within the protoplasm of the cells as a result of the emission of beta rays by the disintegrating phosphorus is not enough to cause significant injury. The concentrations herein employed are well below the threshold value for injury as reported by Mullins (1939) for *Nitella*.

The period allotted for the absorption of the radiophosphorus was fixed at 48 hours because this gave the minimum amount of radioactive material in the smaller buds which was compatible with easy and accurate determination when using the above concentration. After the period of absorption, a subsequent two-day period was allowed in ordinary

¹ Received for publication October 20, 1944.

² The radiophosphorus was very generously supplied by Drs. E. O. and J. H. Lawrence of the Radiation Laboratory, University of California.

nutrient solution in order to permit a complete distribution of the "marked" phosphate throughout the plant. Table 1 will show this distribution period is ample.

TABLE 1. *A preliminary study of the distribution of P^*O_4 in three different plants at various intervals after absorption. Expressed as per cent of the total found in each plant.*

	Plant A	Plant B	Plant C
Abs. period	24 hrs.	48 hrs.	48 hrs.
Distr. period	24 hrs.	48 hrs.	96 hrs.
Leaves	43%	51%	59%
Bark	15	12	6
Wood	17	12	11
Roots	26	26	24

It is evident that distribution is complete and that the developing flowers would have ample opportunity to receive "marked" phosphate via the phloem as well as via the xylem, but it is equally obvious that it is impossible to distinguish that which is delivered via the phloem from that which is delivered via the xylem.

The age of the flower buds with respect to the probable date of meiosis in the micro- and megagametophytes, and of anthesis, had to be known with some certainty. Hence a seven-week study of forty individual buds was undertaken, beginning prior to the experimental period. The growth curves of these buds were plotted individually by taking measurements every one, two, or four days, depending on the size of the buds. From twenty-five of these individual records a composite graph was made. Using this curve, with successive measurements of a given flower bud, it is possible to estimate, with a reasonable degree of accuracy, the number of days until anthesis. This was done for the buds of the experimental plants which were harvested prior to blooming in order that all ages could be recorded with respect to full bloom as a reference point.

Gore (1932) states that in Upland cotton approximately three days elapse between the opening of the first flower on one fruiting branch and the first flower on the next fruiting branch above, and that approximately six days elapse between flowering dates for successive floral buds on the same fruiting branch. Our results are approximately three and one-half, and nine days respectively. Using our preliminary growth curves and our relative flowering dates to assign ages we feel that, in general, our results are accurate to \pm one day.

The average occurrence of microsporogenesis was twenty-three days prior to anthesis. Meiosis in the megagametophyte occurred about twelve days prior to anthesis. This latter was determined by making gross examinations of the ovules in comparison with the descriptions of the process by Gore (1932). Fusion of nuclei at fertilization normally occurs within 32 hours after the opening of the flower

(Gore, 1932). The corolla is dropped from the flower the day after anthesis.

RESULTS.—Three periods of rapid elongation accompany floral development. They are: (1) elongation of bracts beginning prior to microsporogenesis and following a typical S-shaped growth curve; (2) elongation of the flower bud proper, terminating in the very rapid elongation of the corolla just prior to anthesis; and (3) elongation of the ovary, which also follows a typical growth curve with the most rapid period of elongation following fertilization. These curves are shown in figure 1. The stage of development of the flower buds at various ages is also shown in this figure. The numerals under each drawing indicate the number of days until that flower would be in full bloom.

The dry weight data for the flowers or fruits at various ages are presented in figure 2. A study of this curve will show a slight departure from a smoothly ascending curve at the time of megasporogenesis. From the nature of the irregularity it is presumed that it is due to a diversion of the incoming carbohydrate to respiratory channels during this period. During the events of fertilization and immediately afterward a reduced rate of dry weight accumulation is also noted. This is also presumed to be due to a diversion of materials into respiratory channels during the events of fertilization. This is followed, within a few days, by a very rapid gain in dry weight leading into the "grand period of growth" of the fruit, with a maximum increase occurring between the fifteenth and twenty-sixth days after anthesis. No striking demonstration of an effect of microsporogenesis on weight increase was obtained.

The daily increments of phosphate (represented as P^*O_4) to flowers of various ages are shown in figure 3. The increments to the flowers are from that phosphate which was absorbed from the nutrient solution 48 to 96 hours previously.

A study of figure 3 will show certain interesting points in the history of phosphate accumulation by flowers at different ages. (1) The daily increment of phosphate in the developing flowers increases each day until anthesis. The smallest increase is perhaps for those days near the time of megasporogenesis. The spread of individual datum points is wide, rendering exact interpretation difficult, but it appears as though a reduced rate of increase in the daily increment of P^*O_4 occurs at this time. (2) A rapid rate of increase in the daily increment of phosphate accompanies the progressive expansion of the floral bud, terminating in a maximum at the time of anthesis. This is undoubtedly due to an increasing transpiration rate from the expanding corolla and exposed parts, which is responsible for an accelerated delivery of water-soluble phosphate through the connecting xylem channels. (3) Immediately after anthesis the daily increment of phosphate falls to a lower level but increases subsequently.

From the spread of the individual datum points

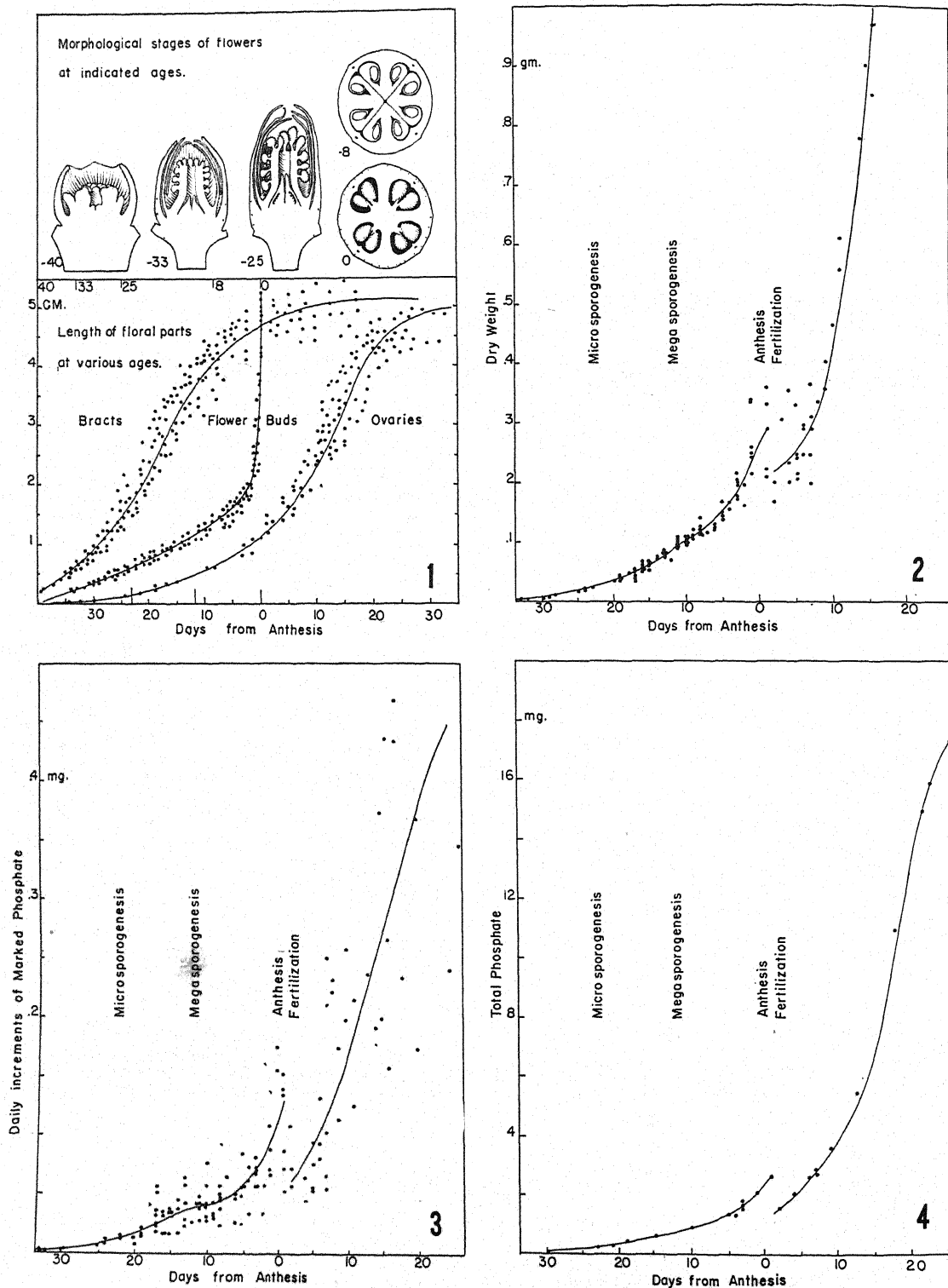


Fig. 1-4.—Fig. 1. The morphological stages of the cotton flowers, and the length of the floral parts, at various ages.—Fig. 2. The dry weights of the flowers or fruits from five individual cotton plants plotted against age.—Fig. 3. The daily increments of marked phosphate in the flowers or fruits from five individual cotton plants.—Fig. 4. The total phosphate content of the flowers or fruits from plant number 5 plotted against age. All ages are expressed as number of days from anthesis.

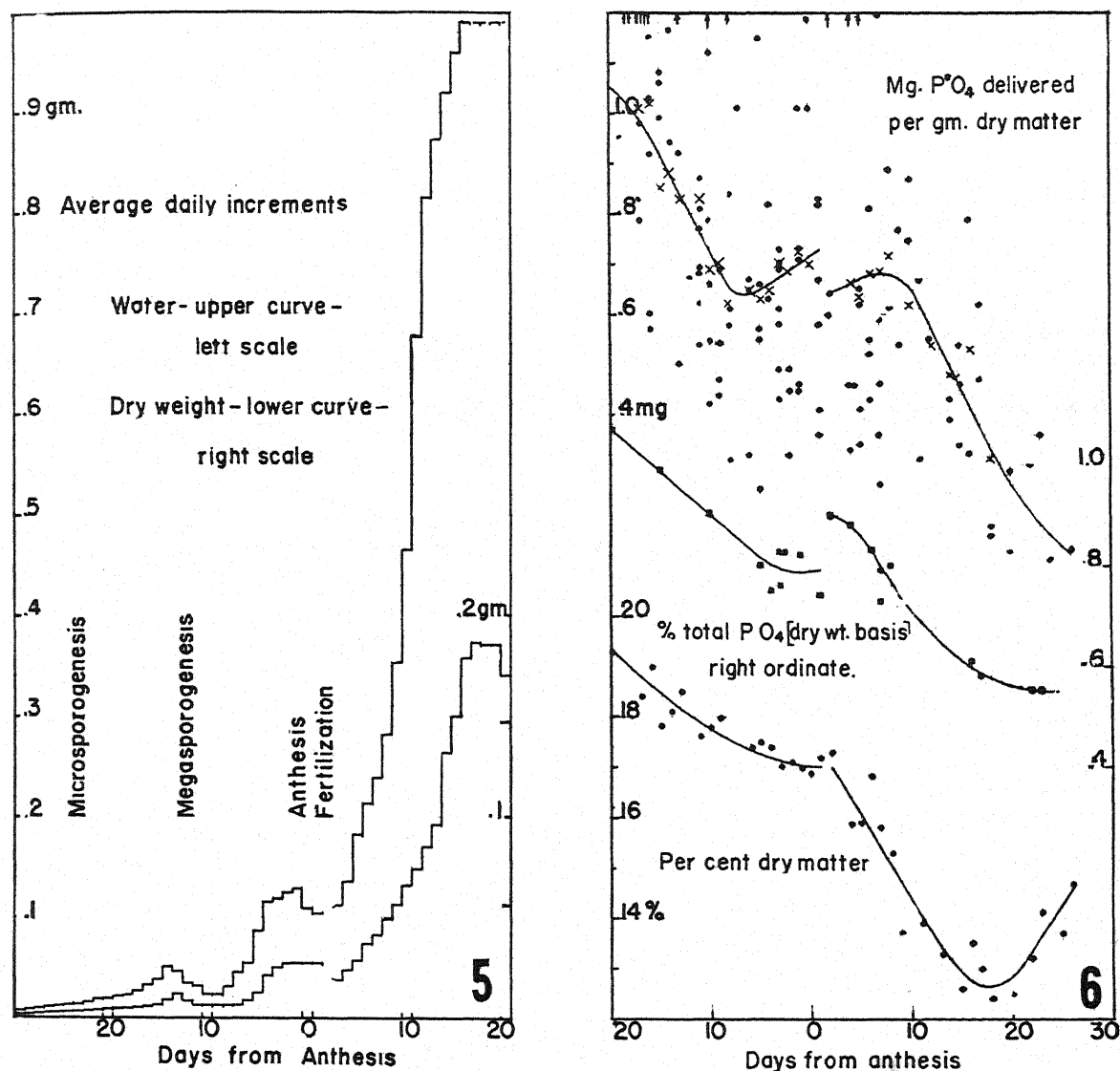


Fig. 5-6.—Fig. 5. The average daily increments of water and of dry weight in the flowers or fruits from five individual cotton plants.—Fig. 6. Upper curve. The quantity of marked phosphate delivered (per gram of dry matter) to the flowers or fruits on five individual cotton plants plotted against age. The crosses represent running averages obtained by averaging each three successive daily means. Each arrow at the top of the graph indicates an individual datum point which fell outside of the limits of the graph.—Middle curve. The per cent (dry weight basis) of total phosphate in the flowers or fruits from plant number 5 plotted against age.—Lower curve. The per cent of dry weight in the flowers or fruits from five individual cotton plants plotted against age. All ages are expressed as number of days from anthesis.

in figure 3, it is apparent that the daily increments of phosphate to flowers or fruits of similar age are quite variable, but a glance at figure 4 will show that the summation of the daily increments, i.e., the total phosphate, is quite uniform, at least for plant number 5. Apparently, over a period of time, the varying daily supplies to individual flowers are "averaged out." Sunlight and shading are presumed to be important factors in accounting for random variations in the daily increments of phosphate.

A graphic representation of the total phosphate

in the flowers of plant number 5 is presented in figure 4.

The average daily increments of dry matter and of water have been calculated from enlarged smoothed curves such as shown in figure 2. These data are presented in figure 5. The daily increments do not increase uniformly with age but show well-marked maxima and minima. The maxima occur at approximately nine days after microsporogenesis, eleven days after megasporeogenesis and sixteen days after fertilization, with well-marked minima occurring between them. The records be-

yond sixteen days are from progressively fewer fruits, but they show that the daily increments of both dry matter and water decrease after this time.

The quantity of marked phosphate delivered per gram of dry matter, the per cent of total phosphate (dry weight basis) and the per cent of dry matter in each flower structure are plotted against the age of the flower in figure 6. These data show that the developing flower primordia possess higher rates of phosphate accumulation and contain higher percentages of phosphate than do the later stages. The same is true for the percentage of dry weight. The latter indicates that hydration of the flower primordia begins early and progresses rather uniformly until fertilization takes place. After fertilization

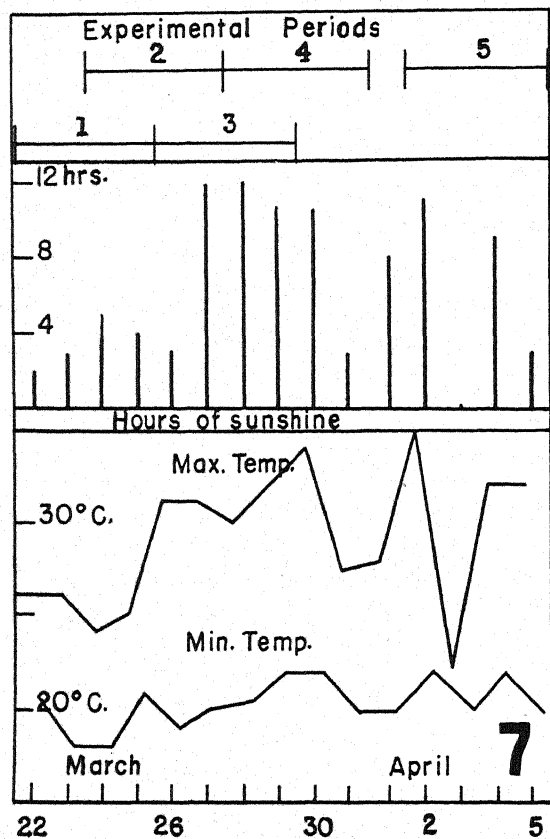


Fig. 7. The environmental conditions existing during the periods of absorption and translocation of the radio-phosphorus. Each plant was allowed to absorb radio-phosphorus for 48 hours; then 48 hours was allowed for its distribution before analyses of flowers were conducted.

a more rapid hydration occurs and it is not until the beginning of the third week that the dehydration of the fruit begins. The two phenomena, that of a high initial phosphorus delivery and consequently a high phosphorus content, and that of a high percentage of dry matter, demonstrate that adequate supplies of both phosphate and carbohydrate are readily available to the young flower primordia in their earliest stages of development.

Figure 7 shows some of the environmental conditions existing during the absorption and migration periods and gives the general plan of rotation of the plants through the experimental periods.

DISCUSSION.—The highest relative rate of marked phosphate accumulation in the developing floral structure and consequently the highest percentage of phosphorus in the tissues, occurs in the young flower primordia. The significance of this point in connection with the state of our present knowledge can best be pointed out by a quotation from Steward (1935).

"The meristematic cell is the logical starting point for any survey upon the relation of growth to active salt accumulation. For strictly meristematic cells there is, unfortunately, little or no precise evidence. By a pardonable extension of the results obtained with other systems (e.g., storage tissues and roots) there is a strong presumption that the intensity of salt accumulation measured by the concentrations obtainable would be at a maximum. Investigation upon the distribution of mineral nutrients (especially potassium, the most readily accumulated cation) indicate their relative abundance in the more active tissues." He then quotes James and Penston (1933) as follows: "There is thus a close connection between abundance of potassium and active growth, but there is no evidence that the potassium precedes and provokes the growth. The alternative that the growing tissues have the capacity for collecting the potassium that they may require for further growth is equally allowable and on the evidence of other lines of work, more probable."

Our results on the meristematic flower tissues render "a pardonable extension of results . . ." less necessary, and show conclusively that in the cotton flower the closer we approach to the purely meristematic cells the higher is the rate of accumulation and, therefore, the per cent of phosphorus in the tissues. Furthermore, our results show a very close if not a causal relationship between synapsis and syngamy on the one hand and dry weight accumulation on the other, but less of a determinate relationship for phosphorus accumulation. We therefore conclude that the growing tissues have the capacity to collect the phosphorus that they may need for further growth, but that the phosphorus does not provoke growth. Hence our results for phosphorus parallel those of James and Penston for potassium.

An observation of interest is the apparent removal of *in situ* phosphorus from the corolla prior to its being dropped. This situation is manifest by a rapid delivery of marked phosphate to the flower prior to anthesis without a corresponding gain in the percentage of total phosphate. Also after the shedding of the corolla the remainder of the flower structure contains a higher percentage of total phosphate than before, which indicates that the corolla, at the time of shedding, was relatively low in phosphate.

Phyllis and Mason (1936) have reported the removal of phosphate from the corolla prior to its being shed, but in citing agreement with their findings the present writers hold certain reservations regarding their statement that the migratory phosphate moves into the corolla the night preceding anthesis and that the phloem is the tissue through which it travels. However, we would agree that removal is most likely to be via the phloem.

Bakhuyzen (1937) has considered in some detail the growth responses which accompany reproduction in annual plants (see especially pp. 57-64). He has analyzed the data from twelve papers in addition to his own for wheat and contends that the following phenomena, at the time of fertilization, are at a minimum: increase in water relative to dry weight, increase in dry weight relative to leaf area, increase in total dry weight, increase in leaf area, growth in length. All minima are said to hold for the plant as a whole as well as for tops, leaves, stems, inflorescences and roots separately.

On a single cotton plant it is not uncommon to find fifty to seventy flowers or fruits representing all stages of development and it is entirely doubtful whether the plant as a whole suffers all of the minima mentioned by Bakhuyzen each time an individual flower passes the fertilization stage. In this respect plants which produce flowers more or less continuously must react differently from those whose flowers all come into bloom more or less simultaneously. Our results indicate that the events of synapsis and syngamy occur at the proper times to be causal agents in producing the observed specific growth responses within the developing cotton flowers. And we believe it is probable that in plants where all flowers develop synchronously similar combined stimuli resulting from the events of synapsis and syngamy are great enough to cause sharply defined responses in the plant as a whole. We offer this suggestion because minima in growth rates occur at the time of syngamy in both plant body and inflorescences in such plants.

Similar suggestions that synapsis and syngamy may be related to growth responses in the plant as a whole come from the calculations of Briggs, Kidd and West (1920) on the data of Kreusler et al. Though these calculations are the subject of considerable criticism (Fisher, 1921; Bakhuyzen, 1937) neither critic has shown that the maxima and minima are entirely a result of the method of calculation. The magnitude of the maxima and minima may be questioned and the exact time of occurrence of each may be uncertain, but the suggestion that the phenomena itself may exist is worthy of consideration. Murneek and Wittwer (1943) and Wittwer (1943) have also presented data which indicate that the events of synapsis and syngamy exert influences which result in measurable responses within the plant as a whole.

In the cotton flower each synaptic or syngamic event precedes a period of rapid growth in the flower or fruit in question; minima occur simulta-

neously with the events of megasporogenesis and fertilization. This leads to the suggestion that a critical examination might disclose a third minimum (during microsporogenesis). Our data for this period, though very limited, suggest that such a minimum might exist. The suggestion is supported by a calculation of the per cent of increase in dry matter per day.

After attempting to relate our results to the findings of others, we wish to point out deficiencies in the work of other authors which have made interpretations difficult. They are (1) failure to include accurate determinations by actual microscopic observation of the morphological stages of the materials in question, and (2) failure to consider the fact that microsporogenesis and megasporogenesis may not occur simultaneously but may be separated by a significant time interval.

SUMMARY

The daily increments of dry matter, water and phosphate to flowers and fruits in varying stages of development have been measured, furnishing a continuous history of the net gain in each fraction as the flowers and fruits mature.

Variations in the size of the daily increments of dry matter were found to follow the events of meiosis and fertilization. Maxima occur at approximately nine days after microsporogenesis, eleven days after megasporogenesis and sixteen days after fertilization. Well-marked minima occur between them.

The daily increments of water to the developing flowers and fruits follow the same general pattern as for dry matter, but the ratio of dry matter gain to water gain is not constant. Calculations of the percentage of dry matter shows a gradual net hydration of the primordia up to the time of fertilization, then a more rapid hydration for approximately two weeks, after which dehydration of the fruit ensues.

The daily increments of marked phosphate to the developing flowers and fruits were influenced to a lesser extent by the events of synapsis and syngamy. Well-marked maxima and minima were absent, but gains appeared after each event.

The per cent of marked phosphate delivered and the per cent of total phosphate present (dry weight basis) were highest in the young primordia, decreasing progressively thereafter until about five days prior to anthesis. Gains, especially in marked phosphate, are then recorded during anthesis. Both fractions decrease after fertilization.

DEPARTMENT OF BOTANY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON

LITERATURE CITED

- BAKHUYZEN, H. L. VAN DESANDE. 1937. Studies on wheat grown under constant conditions. Food Research Inst., Stanford Univ., California.
BRIGGS, G. E., F. KIDD, AND C. WEST. 1920. A quantita-

- tive analysis of plant growth Pt. I. *Ann. of Applied Biol.* 7: 103-123.
- DUNNING, J. R., AND S. M. SKINNER. 1935. Ionizing particle counters. *Rev. Sci. Inst.* 6: 243-246.
- EVANS, R. D., AND R. L. ALDER. 1939. Improved counting rate meter. *Rev. Sci. Inst.* 10: 332-336.
- FISHER, R. A. 1921. Some remarks on the methods formulated in a recent article on "The quantitative analysis of plant growth." *Ann. Applied Biol.* 7: 367-372.
- GORE, U. R. 1932. Development of the female gametophyte and embryo in cotton. *Amer. Jour. Bot.* 19: 795-807.
- JAMES, W. O., AND N. L. PENSTON. 1933. Studies on the physiological importance of the mineral elements in plants. IV. *Ann. Bot.* 47: 279-293 (p. 292).
- LOEHWING, W. F. 1942. Nutritional factors in plant growth and development. *Iowa Acad. Sci.* 49: 61-112.
- MULLINS, L. J. 1939. The effects of radiations from radioactive indicators on the penetration of ions into *Nitella*. *Jour. Gen. and Comp. Physiol.* 14: 403-405.
- MURNEEK, A. E., AND S. H. WITTWER. 1943. Synapsis and syngamy as stimulating processes of plant development. *Science* 98: 384-385.
- PHYLLIS, E., AND T. G. MASON. 1936. Further studies on transport in the cotton plant. VI. Interchange between the tissues of the corolla. *Ann. Bot.* 50: 679-697.
- STEWART, F. C. 1935. Mineral nutrition of plants. *Ann. Rev. Biochem.* 4: 519-544 (p. 528).
- SHIVE, J. W., AND W. R. ROBBINS. 1937. Methods of growing plants in sand and solution cultures. Publications of Rutgers University. Bulletin 636.
- WITTWER, S. H. 1943. Growth hormone production during sexual reproduction of higher plants. Research Bulletin 371, Univ. of Missouri Agric. Exp. Sta.
- ZINZADZE, C. 1935. Colorimetric methods for the determination of phosphorus. *Ind. & Eng. Chem.* 7: 227-230.

RAPID TOTAL EXTRACTION OF AUXIN FROM GREEN PLANT TISSUE¹

G. S. Avery, Jr., J. Berger, and R. O. White

THE OBJECT of the present study was to discover a rapid procedure for the total extraction of auxin from green plant tissues. In the course of this work the presence of auxin precursors was encountered, and the procedure as finally developed converts these precursors to auxin in 30 minutes.

Van Overbeek's adaptation of Boysen Jensen's method of extraction of auxin from plant tissues has been widely used since its presentation. Within the past three to five years, however, increasing difficulties have been encountered with this procedure, at least with many tissues. Thus Thimann and Skoog (1940), Gustafson (1940), Link *et al.* (1940), and others have found that some green tissues, if allowed to remain in ether, continue to yield auxin slowly over long periods of time, even as much as one year. Obviously in these cases the use of ether as an auxin extracting agent is wholly inadequate. A good extraction procedure should rapidly and consistently remove all the existing and potential growth-promoting substances in a given tissue. The presently described procedure fulfills these objectives satisfactorily for green tissues of the Cruciferae.

As regards other work on the extraction of auxin from Cruciferae, Linser (1939, 1940) has described a simple alcohol extraction technique for removal of auxin from green tissues. His data indicate high yields from "hydrakohl" (*Brassica oleracea acephala* D.C.²) and kohlrabi (corresponding to about 20 to 120 micrograms of indoleacetic acid per gm. dry weight, 2 to 12 million TDC) and lesser yields from brussel sprouts, cabbage, etc. In one instance, he calculates an auxin yield from kohlrabi equivalent to 0.02 per cent pure indoleacetic acid, and in another instance 0.06 per cent from hydrakohl (on a fresh weight basis). These extraordinary yields are the highest so far reported in the literature. Link, Eggers and Moulton (1940) report an auxin yield obtained by ether extraction from cabbage equivalent to about 45 micrograms of indoleacetic acid per gram dry weight (4.5 million TDC), but this required 22 hours extraction.

MATERIALS AND METHODS.—Green tissues from a large variety of plants have been extracted and assayed for auxin in this laboratory. Green leaves of the crucifers proved to be especially rich sources of auxin, and after a preliminary survey of half a dozen or more species, cabbage and kohlrabi were selected for more detailed investigation.

Extracts of fresh green leaves from cabbage purchased in the vegetable market were tested for total auxin activity at different seasons of the year. Low yields of total auxin were obtained in many winter samples; the maximum yields were encountered in the green leaves of summer cabbage.

To provide uniform sources of material for detailed experiments, green tissues were dried 24 to 48 hours at low temperature and pressure in an apparatus described by Hays and Koch (1942). They were then ground to a powder and kept at room temperature in a desiccator in the dark until used. Unless otherwise mentioned, the data in this work were obtained on such dried materials. The fresh green leaf tissues were taken from 30 cm. high kohlrabi (*Brassica caulorapa* Pasq.) plants growing in the greenhouse and from the outer leaves of heads of market cabbage (*Brassica oleracea capitata* L.).

"Free" auxin yields are designated as those obtained by extraction of 40 to 50 mg. dried ground tissue with 10 cc. water for 30 minutes at natural

¹ Received for publication December 13, 1944. Work carried out at Connecticut College.

² Correspondence from Dr. Hans Linser, Landw. Versuchsstation, Limburgerhof, Germany, May 8, 1941.

pH and 25°C. "Total" auxin yields (which include free auxin and auxin precursor) are obtained in this study when tissue suspensions in acid or alkali are autoclaved at 120°C. for 30 minutes before auxin assay. The term "precursor" is, as before (Avery, Berger, and Shalucha, 1941), used to designate a compound (or compounds) which is physiologically inactive in the *Avena* test until converted to auxin by suitable treatment, such as alkali or acid hydrolysis. Precursor yield is calculated as the difference between the total and the free auxin. Before *Avena* assay, suspensions are adjusted to about pH 6, and clarified by centrifuging. The clear aqueous extracts are then diluted with water, so that a series of concentrations is available for making up agar blocks for use in *Avena* tests. Final pH values are determined with the glass electrode after treatment of suspensions or extracts.

Skoog's deseeded *Avena* method (cf. Avery, Creighton, and Hock, 1939) was used in this study; all yield data reported have been calculated from *Avena* curvatures shown to be in the proportionality range, so that they are quantitatively significant. Yields are expressed in total degrees curvature ("TDC") per gram dry weight of tissue (cf. Avery, Berger, and Shalucha, 1941). One microgram of indoleacetic acid is equivalent to about 100,000 TDC units.

OBSERVATIONS.—Previous experience with total auxin extraction from maize indicated that the pH is the most important single factor. Therefore, the effect of pH on auxin yield from green tissue was investigated first.

Effect of acid and alkali treatment on auxin yield from cabbage.—In figure 1 are summarized the results obtained when dried cabbage tissue is extracted with buffer solutions over the pH range of 2 to 10, and with stronger acid and alkali solutions. In all cases except the control (unheated, at natural pH of tissue), suspensions are autoclaved 30 minutes at the various pH values indicated. It may be seen that from pH 2 to 5.5 yields are about one-third greater than the untreated control; from pH 6.6 to 10, yields are the same as the control. It can be mentioned that the components of the buffer mixture are not responsible for keeping low the difference in the amount of auxin produced at pH 3 to 10 and that produced with strong acid or alkali, since adjustments with pure acid and alkali over this pH range give the same type of results. The buffer mixture was used to give more controllable pH values. However, treatment with 1 N alkali (NaOH) gives auxin yields that are 6 to 7 times greater than the control, and treatment with 0.5 N acid (HCl) doubles the yield. Thus, both acid and alkali treatments increase auxin yields and, with this particular sample of cabbage, the maximum yield is obtained upon hydrolysis in 1 N alkali. Treatment with higher concentrations of acid or alkali results in decreased yields.

Effect of temperature and time of heating on

auxin yield from cabbage.—From the curves in figure 2 it may be seen that at both 100° and 120°C. yields continue to increase for 20 minutes, and that 30 minutes of heating at 120° gives the maximum yield. Autoclaving in 1 N NaOH for 30 minutes was therefore adopted as standard procedure for total auxin extraction from cabbage.

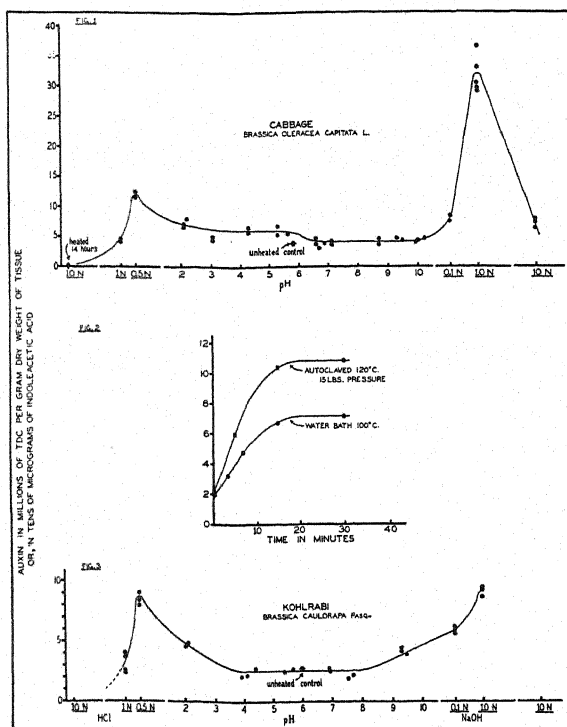


Fig. 1. Effect of pH on auxin yield from cabbage. When suspensions were hydrolyzed in the range of pH 2-10, a single mixed buffer containing KCl-HCl, Na acetate, Na phosphate, Na borate and NaOH was used. In other cases, NaOH or HCl solution was used (see text). Suspensions were autoclaved for 30 minutes.—Fig. 2. Effect of temperature and time of heating on auxin yield from cabbage tissue, in the presence of 1 Normal NaOH. A different batch of dried tissue was used here, from the one used in figure 1, thus accounting for the difference in yield.—Fig. 3. Effect of pH on auxin yield from kohlrabi. In the range of pH 2-10, a buffer of the same composition as in figure 1 was used. Suspensions were autoclaved for 30 minutes.

Effect of acid and alkali on auxin yields from kohlrabi and other crucifers.—The yield data presented in figure 3 show the same general trend as those in figure 1. Thus, autoclaving within the usual pH range does not increase the auxin yield from kohlrabi, but hydrolysis with 0.5 N HCl or 1 N NaOH almost quadruples the yield.

Preliminary tests were made on green leaves of other crucifers (rutabaga, turnip, chinese cabbage and broccoli) prepared for extraction in the same manner as for cabbage and kohlrabi. In every instance, auxin yields from tissue hydrolyzed in 1 N alkali were increased from 2- to 6-fold over the

yields obtained at natural pH. Treatment with 0.5 N acid also increased auxin yields in some instances.

Stability of the liberated auxin.—The data in figures 1, 2 and 3 are a reflection primarily of the conversion to auxin by acid and alkali of the auxin precursors in cabbage and kohlrabi. The data also depend upon the stability of the liberated auxins. To investigate the latter independently, auxin was first liberated from the tissues with 1 N alkali, and then re-heated with acid (table 1). It may be concluded from items (1) and (2) of table 1, that the alkali-liberated auxin from both tissues is acid labile. These properties are similar to those of indoleacetic acid. From items 3 and 4 in table 1, it appears that the acid-liberated auxin is also stable to alkali.

TABLE 1. *Stability of liberated auxin to acid and alkali.*

No.	Treatment	Auxin yield in millions of TDC/gm. dry wt.	
		Cabbage	Kohlrabi
1.	Autoclaved in 1 N sodium hydroxide, 30 minutes	27.6	9.0
2.	(1) re-heated in 1 N hydrochloric acid, 30 minutes	2.1	0.6
3.	Autoclaved in 0.5 N hydrochloric acid, 30 minutes	8.4
4.	(3) re-heated in 1 N sodium hydroxide, 30 minutes	9.1

Solubility of the auxin and auxin precursors.—Preliminary tests have shown that refluxing with absolute alcohol yields an extract from kohlrabi tissue that contains about the same "free" auxin activity as an untreated aqueous extract (1.8 million TDC/gm. vs. 2.3 for water extraction). Thus the free auxin is soluble in alcohol, as one would conclude from the work of Linser. On the other hand, acid and alkali treatments of such an alcohol extract result in only insignificant increases in auxin yield, indicating that the auxin precursors are relatively insoluble in alcohol.

In one experiment, a clear aqueous extract of kohlrabi was made from 50 mg. dry tissue in 5 cc. water, pH 5.8. This was assayed as such, and again after 30 minute acid and alkali hydrolysis. The following auxin yields (in millions of TDC/gm. dry wt.) were obtained: unheated, 2.3; autoclaved in 1 N sodium hydroxide, 4.3; autoclaved in 0.5 N hydrochloric acid, 7.6. These results indicate that some of the auxin precursor (s) is readily soluble in water. And if the data are borne out by further experiments later, the alkali hydrolyzable precursor appears to be less soluble than the acid hydrolyzable one.

DISCUSSION.—The present study has been limited to species of one genus of the Cruciferae, in which it has been found that alkaline as well as acid hydrolyses give increased auxin yields.

Alkaline hydrolysis has been used as a procedure for auxin extraction from kernels of corn and wheat (Avery *et al.*, 1941, 1942, and Haagen-Smit *et al.*, 1942) and from rye (Hatcher, 1943). Gordon and Wildman (1943) showed that alkaline hydrolysis of casein, purified spinach protein and pure tryptophan, gave small yields of auxin, up to about 0.01 per cent of the dry weight. It was suggested by Gordon and Wildman that since tryptophan is undoubtedly present in green tissues, the use of this procedure in auxin extraction is open to question. The significance and extent of conversion of tryptophan to auxin has been discussed elsewhere (Avery and Berger, 1943). It is perhaps sufficient to point out here that many green tissues do not yield auxin on alkali treatment but do undoubtedly contain tryptophan, *i.e.*, in such tissues tryptophan is apparently not converted in sufficient amounts to be measurable in the *Avena* test. Moreover, assuming even a 0.1 per cent conversion of tryptophan to indoleacetic acid on alkali treatment (which is 10 times as high as has been obtained experimentally) then average cabbage samples assaying about 25 million TDC/gm. dry wt. (or 0.025 per cent indoleacetic acid, after conversion) would have to contain 25 per cent tryptophan, which is, of course, extremely unlikely. Young growing points of cabbage have been found to yield as high as 240 million TDC/gm. dry wt. or 0.24 per cent indoleacetic acid on treatment with 1 N sodium hydroxide (Avery and Pottorf, 1945).

That the auxin extraction method set forth in this study is applicable to at least certain other green tissues than those of the Cruciferae, is indicated in a communication from a former associate, Miss Barbara Shalucha. She reports extractions from young leaves and growing points of peach (*Prunus persica* Sieb. & Zucc. var. South Haven) as giving auxin yields of at least 20 million TDC per gram dry weight of tissue (1 N NaOH, 60 minutes at 120°C.).

Link, Eggers and Moulton (1940) report that the auxin in ether extracts of cabbage is resistant to acid and alkali, but since none of their data (table 4 of their paper) on this point are significant, *i.e.*, no *Avena* curvatures are in the proportionality range, their evidence is insufficient for this conclusion.

The data in the present study clearly show that not only is the major part of the total auxin in cabbage stable to autoclaving in reasonably strong alkali (1.0 N) and acid (0.5 N but not 1.0 N), but indeed hydrolysis with these reagents gives maximum auxin yields. These maximum auxin yields are composed of both the naturally occurring free auxin and the converted auxin precursor (s). The stability of the auxin which exists free initially was not determined in these experiments. The stability to 1 N alkali of one of the liberated auxins and its lability in 1 N acid are similar to the properties of indoleacetic acid.

The presence of at least two auxins and two auxin precursors in the Cruciferae is suggested, but

not proven, by the following: (a) Two optima (acid and alkali) exist for the conversion of the precursor(s). Unpublished evidence is on hand from isolation work that the alkali-produced auxin is probably indoleacetic acid; however, the acid-produced auxin cannot be indoleacetic acid, since this is destroyed by acid treatment; (b) the varying ratios of conversion of precursor (in the different species) by 0.5 N hydrochloric acid *vs.* 1 N sodium hydroxide solution. For example, 0.5 N acid liberates the same amount of auxin as does 1 N alkali from kohlrabi (figure 3), only one-third as much from cabbage (figure 1). If only one precursor existed, it would be reasonable to expect a more or less constant conversion ratio.

SUMMARY

In the search for a rapid method of total extrac-

tion of auxin from green plants, it has been found that green tissue from certain species of Cruciferae gives the maximum auxin yield upon autoclaving for 30 minutes in 1 N alkali. In cabbage and kohlrabi, about 20 to 30 per cent of the total auxin is free, and extractable with water alone; the remaining 70 to 80 per cent is precursor, which is at least partly water soluble, and convertible to auxin upon alkaline hydrolysis. Green leaf tissue from these plants contains total auxin equivalent to from 0.03 to 0.35 mg. indoleacetic acid per gm. of dry tissue.

Hydrolysis of green tissue with 0.5 N acid also gives increased total auxin yields, which in the case of kohlrabi, is equal to that obtained upon alkaline hydrolysis.

BROOKLYN BOTANIC GARDEN,
BROOKLYN, NEW YORK

LITERATURE CITED

- AVERY, G. S., JR., AND J. BERGER. 1943. Tryptophan and phytohormone precursors. *Science* 98: 513-515.
- , —, AND B. SHALUCHA. 1941. The total extraction of free auxin and auxin precursor from plant tissue. *Amer. Jour. Bot.* 28: 596-607.
- , —, AND —. 1942. Total auxin extraction from wheat. *Amer. Jour. Bot.* 29: 612-616.
- , H. B. CREIGHTON, AND C. HOCK. 1939. A low cost chamber for phytohormone tests. *Amer. Jour. Bot.* 26: 360-365.
- , AND L. POTTORF. 1945. Polyploidy and auxin content of green tissue. (In press.)
- GORDON, S. A., AND S. G. WILDMAN. 1943. The conversion of tryptophan to a plant growth substance by conditions of mild alkalinity. *Jour. Biol. Chem.* 147: 389-398.
- GUSTAFSON, F. G. 1940. Some difficulties encountered in the extraction of growth hormones from plant tissues. *Science* 92: 266-277.
- HAAGEN-SMIT, A. J., W. D. LEECH, AND W. R. BERGREN. 1942. The estimation, isolation, and identification of auxins in plant material. *Amer. Jour. Bot.* 29: 500-506.
- HATCHER, E. S. J. 1943. Auxin production during development of the grain in cereals. *Nature* 151: 278-279.
- HAYS, E. E., AND F. C. KOCH. 1942. An apparatus for vacuum drying in the frozen state. *Science* 95: 633.
- LINK, G. K. K., V. EGGERS, AND J. E. MOULTON. 1940. Avena coleoptile assay of ether extracts of aphids and their hosts. *Bot. Gaz.* 101: 928-939.
- , —, AND —. 1941. Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs: its extraction with dry ether. *Bot. Gaz.* 102: 590-601.
- LINSER, H. 1939. Zur Methodik der Wuchsstoffbestimmung. II. Die Extraktion von Pflanzenmaterial. *Planta* 29: 392-408.
- , —. 1940. Über das Vorkommen von Hemmstoff in Pflanzenextrakten, sowie über das Verhältnis von Wuchsstoffgehalt und Wuchsstoffabgabe bei Pflanzen oder Pflanzenteilen. *Planta* 31: 32-59.
- OVERBEEK, J. VAN. 1938. A simplified method for auxin extraction. *Proc. Nat. Acad. Sci.* 24: 42-46.
- THIMANN, K. V., AND F. SKOOG. 1940. The extraction of auxin from plant tissues. *Amer. Jour. Bot.* 27: 951-960.

A LIST OF CHROMOSOME NUMBERS IN HIGHER PLANTS.

II. MENISPERMACEAE TO VERBENACEAE¹

Wray M. Bowden²

IN A previous paper (Bowden, 1945)³ the chromosome numbers of many angiospermous species were reported and figured. In the present paper chromosome numbers are recorded for numerous species and genera in nineteen additional families of angiosperms and in one gymnospermous family.

CHROMOSOME NUMBERS.—*Menispermaceae*.—No.

¹ Received for publication October 2, 1944.

² Formerly Research Fellow in Agricultural Biology of The Blandy Experimental Farm, University of Virginia, Boyce, Virginia.

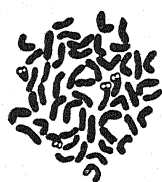
³ In the first line on page 91 of Bowden (1945), (2n = 16, Bowden, 1940a) should have read (2n = 46, Bowden, 1940a).

2831-39, fig. 121. *Cocculus laurifolius* DC., Royal Palm Nursery, Oneco, Florida, 2n = 26.—No. 866-32, fig. 122. *Cocculus trilobus* (Thunb.) DC., pistillate plant, B.P.I., P.I. No. 82420, col. near top of Mt. Koka, Island of Kokato, 2n = 52 (n = 25, Nakajima, 1937, in a staminate plant).—No. 6648-38, fig. 123. *Cocculus carolinus* (L.) DC., G. W. Park Seed So., South Carolina, 2n = 78.—No. 867-37, *Cocculus carolinus* (L.) DC., col. by O. E. White near Lake Waccamaw, North Carolina, 2n = 78.

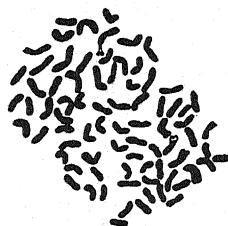
Moraceae.—No. 669-1860, fig. 124. *Broussonetia papyrifera* (L.) Vent., staminate tree, near The



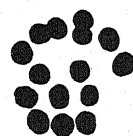
121



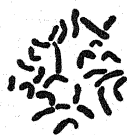
122



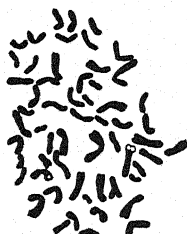
123



124



125



126



127



128



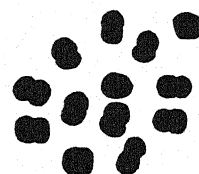
129



130



131



132



133



134



135



136



137



138



139



140

Fig. 121-140.—Fig. 124, 132, 133. Polar views of meiotic stages.—Other figures, polar views of mitotic metaphases.—Magnification $\times 2500$, except figures 124 and 132 which have a magnification $\times 3240$.—Fig. 121-123. Menispermaceae.—Fig. 121. *Cocculus laurifolius* DC., $2n = 26$.—Fig. 122. *Cocculus trilobus* (Thunb.) DC., $2n = 52$.—Fig. 123. *Cocculus carolinus* (L.) DC., $2n = 78$.—Fig. 124, 125. Moraceae.—Fig. 124. *Broussonetia papyrifera* (L.) Vent.,

Quarters, The Blandy Experimental Farm, Virginia, $n = 13$ ($2n = 26$, Bowden, 1940a).—No. 671-36, fig. 125. *Broussonetia papyrifera* (L.) Vent., pistillate tree, near Main Street and Jefferson Park Road, Charlottesville, Va., $2n = 26$.

Nyctaginaceae.—No. 6624-39, fig. 126. *Oxybaphus nyctagineus* (Michx.) Sweet., B.G., Cambridge, England, $2n = 58$.—No. 6688-39, fig. 127. *Oxybaphus viscosus* L'Her., B.G., Coimbra, Portugal, $2n = 58$.

Oleaceae.—No. 2185-39, fig. 128. *Menodora scoparia* Engelm., S.C.S., Arizona, col. Payson, Arizona, $2n = 22$.—No. 5978-40, fig. 129. *Jasminum Mesnyi* Hance., Kiyono Nursery, Alabama, $2n = 24$.—No. 5742-39, fig. 130. *Jasminum officinale* L. var. *affine* (Lindl.) Nichols., B.G., Coimbra, Portugal, $2n = 26$.—No. 2342-33, fig. 131. *Jasminum Beesianum* Forrest. and Diels., B.P.I., P.I. No. 82368, $2n = 26$.—No. 2343-36, fig. 132. *Jasminum floridum* Bunge., LeMac Nursery, Virginia, $n = 13$.—No. 2020-39, fig. 133. *Jasminum officinale* L. var. *grandiflorum* (L.) Kobuski., W. B. Clarke and Son, Calif., $n = 13$.—No. 5743-38, fig. 134. *Jasminum humile* L., Lloyd B.G., Darjeeling, India, $2n = 26$.—No. 6727-39, fig. 135. *Jasminum odoratissimum* L., B.G., Coimbra, Portugal, $2n = 26$.—No. 5744-38, fig. 136. *Jasminum officinale* L., Lloyd B.G., Darjeeling, India, col. 4,000 ft., $2n = 26$.—No. 6279-40, fig. 137. *Jasminum Parkeri* Dunn., Green Past. Gardens, Washington, $2n = 26$.—No. 6935-40, fig. 138. *Jasminum Sambac* Ait. var. *Grand Duke* Hort., Royal Palm Nursery, Oneco, Fla., $2n = 26$.—No. 5745-39, fig. 139. *Jasminum humile* L. var. *glabrum* (DC.) Kobuski., B.G., Copenhagen, Denmark, $2n = 26$.—No. 2344-30, fig. 140. *Jasminum nudiflorum* Lindl., O. E. White, Charlottesville, Va., $2n = 52$.—No. 7602-40, *Jasminum nudiflorum* Lindl., University of Virginia Grounds, $2n = 52$.—No. 5724-38, fig. 141. *Frazinus floribunda* Wall., Lloyd B.G., Darjeeling, India, col. 6,000 ft., $2n = 46$.

Palmaceae.—No. 6850-39, fig. 142. *Sabal causiarum* Becc., E. West, Gainesville, Florida, $2n = 36$.—No. 6845-39, fig. 143. *Sabal minor* (Jacq.) Pers., E. West., Gainesville, Florida, $2n = 36$.—No. 7166-39, fig. 144. *Sabal Palmetto* Lodd., Municipal Park, Southport, North Carolina, from a transplant from the North Carolina coast, $2n = 36$.—No. 6844-39, *Sabal Palmetto* Lodd., E. West, Gainesville, Florida, $2n = 36$.

Papaveraceae.—No. 5711-27, fig. 145. *Chelidonium majus* L., col. by O. E. White in Clarke County, Virginia, $n = 6$ ($n = 6$, Marchal, 1920;

Sugiura, 1936; Winge, 1917); ($n = 8$, v. Boenicke, 1911); ($2n = 10$, Nagao and Sakai, 1939).—No. 5748-28, fig. 146. *Macleaya cordata* R. Br., "Scaleby," Clarke County, Virginia, $n = 10$ ($n = 10$, Sugiura, 1935).—No. 5747-39, fig. 147. *Macleaya cordata* R. Br., Thompson and Morgan, Ipswich, England, $2n = 20$.

Passifloraceae.—No. 4333-39, fig. 148. *Passiflora bryonoides* H. B. and K., B.G., Coimbra, Portugal, $2n = 12$.—No. 7753-39, fig. 149. *Passiflora capsularis* L. var. *acutiflora* Hort., B.G., Stockholm, Sweden, $2n = 12$.—No. 4335-38, fig. 150. *Passiflora coerulea* L., garden at Brantford, Ontario, $2n = 18$ ($2n = 18$, Heitz, 1926) ($n = 9$, Nakajima, 1931; Simonet and Miedzyrzecski, 1932).—No. 4339-34, fig. 151. *Passiflora incarnata* L., col. by O. E. White in Georgia, $2n = 18$.—No. 170-38, fig. 152. *Passiflora mollissima* (H. B. and K.) Bailey., Rex D. Pearce, N.J., $2n = 18$.—No. 4225-39, fig. 153. *Passiflora racemosa* Brot., Henry A. Dreer, Pa., $2n = 18$ ($n = 9$, $2n = 18$, Heitz, 1926).—No. 6680-39, fig. 154. *Passiflora gracilis* Jacq., B.G., Stockholm, Sweden, $2n = 20$.—No. 4336-39, fig. 155. *Passiflora foetida* L., B.G., Singapore, Straits Settlements, $2n = 22$.—No. 4340-37, fig. 156. *Passiflora lutea* L., col. by O. E. White, native, Clarke County, Virginia, $2n = 84$.

Pinaceae.—No. 2202-39, fig. 157. *Pinus canariensis* C. Smith., S.C.S., Santa Paula, California, col. at Corralitas, California, $2n = 24$.—No. 227-38, *Pinus patula* Schlecht. and Chamisso., Katzenstein and Co., Georgia, $2n = 24$.

Portulacaceae.—No. 5684-36, fig. 158. *Talinum* sp., received as *T. Mengesii* Wolf., col. near Tuscaloosa, Alabama, by A. V. Beatty, $2n = 48$.—No. 5687-35, fig. 159. *Talinum teretifolium* Pursh., wild near Wake Forest, North Carolina, col. by H. M. Phillips, $2n = 48$ ($2n = 48$ in two collections, and $2n = 24$ in one collection, Steiner, 1944).

Saxifragaceae.—No. 1880-38, fig. 160. *Itea ilicifolia* Oliver., W. B. Clarke and Son, California, $n = 11$.

Pontederiaceae.—No. 3462-39, fig. 161. *Eichhornia Martiana* Seub., Wm. Tricker, Inc., N.J., $n = 8$.—No. 6599-40, fig. 162. *Eichhornia azurea* Kunth., Henry A. Dreer, Pa., $2n = 32$.—No. 5551-39, fig. 163. *Pontederia cordata* L., col. by W. M. Bowden near Wilmington, North Carolina, $n = 8$ ($n = 8$, R. W. Smith, 1898).—No. 6603-40, fig. 164. *Pontederia cordata* L. var. *angustifolia* Torr., Johnson Water Gardens, Hynes, California, $n = 8$.—No. 6603-40, fig. 165. *Pontederia cordata* L. var. *angustifolia* Torr., see preceding collection;

Metaphase I, $n = 13$.—Fig. 125. *Broussonetia papyrifera* (L.) Vent., $2n = 26$.—Fig. 126, 127. *Nyctaginaceae*.—Fig. 126. *Oxybaphus nyctagineus* (Michx.) Sweet., $2n = 58$.—Fig. 127. *Oxybaphus viscosus* L'Her., $2n = 58$.—Fig. 128-140. *Oleaceae*.—Fig. 128. *Menodora scoparia* Engelm., $2n = 22$.—Fig. 129. *Jasminum Mesnyi* Hance., $2n = 24$.—Fig. 130. *Jasminum officinale* L. var. *affine* (Lindl.) Nichols., $2n = 26$.—Fig. 131. *Jasminum Beesianum* Forrest. and Diels., $2n = 26$.—Fig. 132. *Jasminum floridum* Bunge., Anaphase I, $n = 13$.—Fig. 133. *Jasminum officinale* L. var. *grandiflorum* (L.) Kobuski., Metaphase I, $n = 13$.—Fig. 134. *Jasminum humile* L., $2n = 26$.—Fig. 135. *Jasminum odoratissimum* L., $2n = 26$.—Fig. 136. *Jasminum officinale* L., $2n = 26$.—Fig. 137. *Jasminum Parkeri* Dunn., $2n = 26$.—Fig. 138. *Jasminum Sambac* Ait. var. *Grand Duke* Hort., $2n = 26$.—Fig. 139. *Jasminum humile* L. var. *glabrum* (DC.) Kobuski., $2n = 26$.—Fig. 140. *Jasminum nudiflorum* Lindl., $2n = 52$.



141



142



143



144



145



146



147



148



149



150



151



152



153



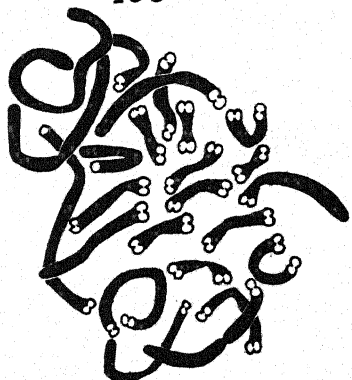
154



155



156



157



158



159



160

Fig. 141-160.—Fig. 145, 146, and 160. Polar views of meiotic stages.—Other figures, polar views of mitotic metaphases.—Magnification, $\times 2500$, except figures 145, 146, and 160, which have a magnification $\times 3240$.—Fig. 141. Oleaceae.—Fig. 141. *Fraxinus floribunda* Wall., $2n = 46$.—Fig. 142-144. Palmaceae.—Fig. 142. *Sabal causiarum* Becc., $2n = 36$.—Fig. 143. *Sabal minor* (Jacq.) Pers., $2n = 36$.—Fig. 144. *Sabal Palmetto* Lodd., $2n = 36$.—Fig. 145-147. Papaveraceae.—Fig. 145. *Chelidonium majus* L., Metaphase I, $n = 6$.—Fig. 146. *Macleaya cordata* R. BR.,

$2n = 16$.—No. 7513-40, fig. 166. *Heteranthera dubia* (Jacq.) MacM., col. by W. M. Bowden near St. Williams, Norfolk County, Ontario, $2n = 30$.

Rhamnaceae.—No. 5710-36, fig. 167. *Ceanothus americanus* L., col. by O. E. White, in Clarke County, Virginia, $n = 12$.—No. 2100-39, fig. 168. *Ceanothus arboreus* Greene., S.C.S., Santa Paula, California, $2n = 24$.—No. 2308-39, fig. 169. *Rhamnus californica* Eschsch., S.C.S. Nursery, Albuquerque, New Mexico, $2n = 24$.—No. 6673-39, fig. 170. *Rhamnus utilis* Dene., Boyce Thompson Arboretum, N.Y., $2n = 24$.—No. 6748-39, fig. 171. *Sageretia theezans* Brongn., U.S.D.A. through H. A. Senn, C.E.F., Ottawa, $2n = 24$.—No. 4171-31, fig. 172. *Zizyphus Jujuba* Mill., B.P.I., P.I. No. 38245, spiny suckers from stock, $n = 12$ ($n = 20$, Srinivasachar, 1940).—No. 4172-31, fig. 173. *Zizyphus Jujuba* Mill. var. *inermis* (Bge.) Rehd. (var. "Shui mèn tsao" Hort.), B.P.I., P.I. No. 38245, scion, variety cultivated near Pailhsiangchen, Shansi, China, $n = 12$ ($n = 12$, Morinaga *et al.*, 1929).—No. 4170-35, *Zizyphus Jujuba* Mill. var. *inermis* (Bge.) Rehd. (var. Lang Hort.), Ramsey's Austin Nursery, Texas, $n = 12$.

Rosaceae.—No. 3758-39, fig. 174. *Quillaja brasiliensis* Mart., B.G., Montevideo, Uruguay, $2n = 34$.—No. 3590-34, fig. 175. *Spiraea tomentosa* L. var. *alba* West., col. by A. Lorz near Allegheny, Pennsylvania, $n = 18$.

Rutaceae.—No. 1050-34, fig. 176. *Dictamnus albus* L., Bobbink and Atkins, N.J., $n = 18$.—No. 5452-31, fig. 177. *Evodia Daniellii* (Benn.) Hemsl., B.P.I., P.I. No. 93115, $n = 36$.—No. 88-38, fig. 178. *Evodia fraxinifolia* Hook f., Lloyd B.G., Darjeeling, India, col. at 5,000 ft., $2n = 72$.—No. 6839-39, fig. 179. *Evodia hupehensis* Dode., B.G., Budapest, Hungary, $2n = 72$.—No. 1900-38, fig. 180. *Zanthoxylum Clava-Herculis* L., col. by O. E. White at Holden Beach, North Carolina, $2n = ca. 72$.

Salicaceae.—No. 2031-39, fig. 181. *Salix Humboldtiana* Willd., Atkins Inst., Arnold Arboretum, Cuba, $2n = 38$.—No. 5758-40, fig. 182. *Salix Bonplandiana* H.B. and K., col. by J. N. Couch in Mexico City, Mexico, $2n = 42$.—No. 3507-33, fig. 183. *Salix babylonica* L., pistillate plant near Millwood, Virginia, cuttings collected by O. E. White, $2n = 76$.

Sapindaceae.—No. 6621-39, fig. 184. *Cardiospermum Halicacabum* L., B.G., Coimbra, Portugal, $2n = 22$ ($n = 11$, Sugiura, 1931).—No. 6057-40, fig. 185. *Koelreuteria formosana* Hayata., Royal Palm Nursery, Oneco, Florida, $2n = 22$.—No. 2392-28, fig. 186. *Koelreuteria paniculata* Laxm.,

University of Virginia Grounds, $2n = 22$ ($2n = 30$, Eichhorn and Franquet, 1936).

Tamaricaceae.—No. 3645-34, fig. 187. *Tamarix gallica* L., col. by O. E. White at Wrightsville Beach, N.C., $n = 12$.—No. 3649-29, fig. 188. *Tamarix hispida* Willd., Storrs and Harrison, Ohio, $n = 12$.—No. 3650-29, fig. 189. *Tamarix odessana* Stev., Storrs and Harrison, Ohio, $n = 12$.—No. 3651-30, fig. 190. *Tamarix parviflora* DC., Munson Nursery, Texas, $n = 12$.—No. 3646-36, fig. 191. *Tamarix parviflora* DC., Boyce Thompson Arboretum, Yonkers, New York, $2n = 24$.—No. 6448-39, *Tamarix parviflora* DC., University of Virginia Grounds, $2n = 24$.—No. 2770-39, fig. 192. *Tamarix pentandra* Pall., Hardy Plant Nursery, Dropmore, Man., $2n = 24$.—No. 7597-40, fig. 193. *Tamarix aphylla* (L.) Karst., W. S. Flory, College Station, Texas, $2n = 24$.—No. 7598-40, *Tamarix aphylla* (L.) Karst., Ramsey's Austin Nursery, Texas, $2n = 24$.

Ulmaceae.—No. 377-37, fig. 194. *Celtis laevigata* Willd., col. by O. E. White at Williamsburg, Va., $2n = 20$.—No. 766-27, fig. 195. *Celtis occidentalis* L., native to The Blandy Experimental Farm, Boyce, Clarke County, Virginia, $2n = 20$.—No. 6586-40, fig. 196. *Celtis sinensis* Pers., Kohankie and Sons, Ohio, $2n = 20$.—No. 6637-39, fig. 197. *Celtis australis* L., B.G., Montevideo, Uruguay, $2n = 40$.

Umbelliferae.—No. 5721-27, fig. 198. *Eryngium maritimum* L., Brooklyn B.G., N.Y., $n = 8$ ($n = 8$, Wulff, 1937).—No. 405-37, fig. 199. *Eryngium aquaticum* L., col. by A. V. Beatty, Tuscaloosa, Alabama, $n = 48$ ($2n = 96$, Wanscher, 1933).—No. 2782-39, *Eryngium aquaticum* L., col. by W. M. Bowden near Chapel Hill, North Carolina, $2n = 96$.

Verbenaceae.—No. 6734-39, fig. 200. *Clerodendron speciosissimum* Van Beert. (= *C. fallax* Lindl.), B.G., Edinburgh, Scotland, $2n = 48$.—No. 2829-39, fig. 201. *Clerodendron Thomsoniae* Balfour. var. *delectum* Hort., Royal Palm Nursery, Oneco, Florida, $2n = ca. 48$.—No. 2830-39, fig. 202. *Clerodendron Kaempferi* (Jacq.) Sieb. (= *C. squamatum* Vahl.), Royal Palm Nursery, Oneco, Florida, $2n = 60$.—No. 860-28, fig. 203. *Clerodendron trichotomum* Thunb., Brooklyn B.G., N.Y., $n = ca. 46$.—No. 6447-39. *Clerodendron trichotomum* Thunb., a horticultural variety distinct from the preceding collection, col. by O. E. White in the Coker Arboretum, Chapel Hill, North Carolina, $2n = ca. 92$.—No. 6722-39, fig. 204. *Clerodendron Bungei* Steud., B.G., Antwerp, The Netherlands, $2n = 108$.

Anaphase I, $n = 10$.—Fig. 147. *Macleaya cordata* R. Br., $2n = 20$.—Fig. 148-156. *Passifloraceae*.—Fig. 148. *Passiflora bryonoides* H.B. and K., $2n = 12$.—Fig. 149. *Passiflora capsularis* L. var. *acutiflora* Hort., $2n = 12$.—Fig. 150. *Passiflora coerulea* L., $2n = 18$.—Fig. 151. *Passiflora incarnata* L., $2n = 18$.—Fig. 152. *Passiflora mollissima* (H.B. and K.) Bailey., $2n = 18$.—Fig. 153. *Passiflora racemosa* Brot., $2n = 18$.—Fig. 154. *Passiflora gracilis* Jacq., $2n = 20$.—Fig. 155. *Passiflora foetida* L., $2n = 22$.—Fig. 156. *Passiflora lutea* L., $2n = 84$.—Fig. 157. *Pinaceae*.—Fig. 157. *Pinus canariensis* C. Smith., $2n = 24$.—Fig. 158, 159. *Portulacaceae*.—Fig. 158. *Talinum* sp., $2n = 48$.—Fig. 159. *Talinum teretifolium* Pursh., $2n = 48$.—Fig. 160. *Saxifragaceae*.—Fig. 160. *Itea ilicifolia* Oliver., Metaphase I, $n = 11$.



161



162



163



164



165



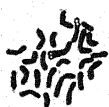
166



167



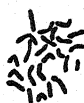
168



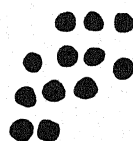
169



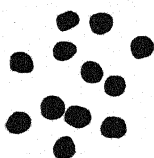
170



171



172



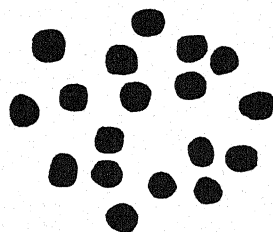
173



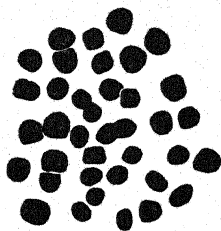
174



175



176



177



178



179



180

Fig. 161-180.—Fig. 161, 163, 164, 167, 172, 173, 175, 176, 177. Polar views of meiotic stages.—Other figures, polar views of mitotic metaphases.—Magnification $\times 2500$, except figures 167, 172, 173, 175, 176, 177, which have a magnification $\times 3240$.—Fig. 161-166. Pontederiaceae.—Fig. 161. *Eichhornia Martiana* Seub., Metaphase I, $n = 8$.—Fig. 162. *Eichhornia azurea* Kunth., $2n = 32$.—Fig. 163. *Pontederia cordata* L., Metaphase I, $n = 8$.—Fig. 164. *Ponte-*

ADDITIONAL CYTOLOGICAL NOTES.—The chromosome number was found to be doubled in small sectors of root tips of the following collections: No. 5727–38, *Helianthemum canum* Boiss., $2n = 44$ in tetraploid sector; No. 4319–38, *Opuntia compressa* Macbride., $2n = 44$ in tetraploid sector; No. 4317–38, *Opuntia impedita* Small., $2n = 88$ in octoploid sector; No. 6727–39, *Jasminum odoratissimum* L., $2n = 52$ in tetraploid sector. Ervin (1941) reviewed the occurrence of polysomaty which has been observed in species of widely-separated families of higher plants. In *Cucumis melo*, all mitoses were monosomatic in the anterior part of the meristem of the root tip, while in older regions polysomatic cells were present in a percentage of cells. During the present investigation, all serial sections were examined and all portions of the tissues were observed for variation in chromosome number.

In serial sections of the anthers of *Asimina triloba* (L.) Dunal., numerous polyploid metaphase plates were observed in the tapetal layer. These polyploid metaphases were mostly tetraploid, $2n = 36$, but a few were octoploid, $2n = 72$, and proved to be valuable for confirming the diploid chromosome-number determination. Darlington (1937) stated that tetraploid and octoploid nuclei are regularly found in the tapetal tissue surrounding the pollen mother cells. Cooper (1933) observed tetraploid and octoploid nuclei occasionally in the tapetal cells of certain angiospermous species.

The cytological characteristics of certain genera and species made them especially favorable for examination. The pollen mother cells of *Lobelia Cardinalis* L. subsp. *Cardinalis* McVaugh proved to be the best material for meiotic stages. The anthers could be smeared well; large numbers of pollen mother cells in each anther were at the same stage of meiosis; and the chromosomes of the metaphase I plates were large enough and well separated so that the bivalents could be counted at relatively low magnifications. The inflorescence was a spike, and, with experience in smearing, a particular flower could be selected from the spike for a stage of meiosis desired at definite times during the day. The best mitotic metaphase material was found in root tips of species of Annonaceae, and in species of *Sisyrinchium*, *Helianthemum*, *Hydrophyllum*, *Jasminum*, *Passiflora*, and *Tamarix*. Several genera and families which require cyto-taxonomic investigation and which would be suitable for detailed cytological examination were noted, namely: the genera *Lobelia*, *Opuntia*, *Sassafras*, *Sisyrinchium*,

Helianthemum, *Passiflora*, and the family Annonaceae.

The genus *Helianthemum* is much confused taxonomically and there is evidence to indicate that data on chromosome number and morphology would aid the taxonomy of this genus. Two series of chromosome numbers have been reported by Chiarugi (1925) and Bowden (1945) for different collections of species of *Helianthemum*, namely, $2n = 16, 32, 48$; and $2n = 20, 22$. The species of *Helianthemum* reported in the present list were determined by the botanical gardens which supplied the seeds except in the case of a few collections which were re-identified. Several synonymous names have been listed since it was clear that any attempt to refer certain species to better-recognized species was not justified by the cytological data. An extensive cytological and taxonomic survey of this genus is required.

DISCUSSION.—*Cytological technique*.—While both aceto-carminic smears of pollen mother cells and stained serial sections of root tips, young leaves, stem tips, and young flower buds were used during this investigation, the chromosome complement was defined best in stained serial sections of root-tip material. Aceto-carminic smears provided a rapid method for ascertaining chromosome number and demonstrated the pairing relationships of homologous chromosomes at Metaphase I and the morphology of the chromosomes of the haploid complement at Metaphase II. However, routine examinations of mitotic metaphases in rapidly-proliferating somatic tissue, preferably in root tips, yielded much more information on chromosome morphology (compare fig. 13 with fig. 14, and fig. 164 with fig. 165). Darlington (1937, see p. 71) has emphasized the value of the examination of meiotic stages when detailed information on the composition of the chromosome complement is required.

Chromosome size is known to vary between individuals in a population of the same species, as well as between larger taxonomic units. Darlington (1937) has discussed the problem of chromosome-size differences in sister cells, individuals, and races, and in larger units. During this survey, it was noted that chromosome size varied in different tissues and organs. The largest somatic chromosomes and the largest metaphase plates for an individual plant were observed in the largest cells in the root tips while the smallest chromosomes were observed in somatic cells in young flower buds in which cell size was correspondingly smaller. The

deria cordata L. var. *angustifolia* Torr., Metaphase I, $n = 8$.—Fig. 165. *Pontederia cordata* L. var. *angustifolia* Torr., $2n = 16$.—Fig. 166. *Heteranthera dubia* (Jacq.) MacM., $2n = 30$.—Fig. 167–173. Rhamnaceae.—Fig. 167. *Ceanothus americanus* L., Metaphase I, $n = 12$.—Fig. 168. *Ceanothus arboreus* Greene., $2n = 24$.—Fig. 169. *Rhamnus californica* Eschsch., $2n = 24$.—Fig. 170. *Rhamnus utilis* Dene., $2n = 24$.—Fig. 171. *Sageretia theezans* Brongn., $2n = 24$.—Fig. 172. *Zizyphus Jujuba* Mill., Metaphase I, $n = 12$.—Fig. 173. *Zizyphus Jujuba* Mill. var. *inermis* (Bge.) Rehd., Metaphase I, $n = 12$.—Fig. 174, 175. Rosaceae.—Fig. 174. *Quillaja brasiliensis* Mart., $2n = 34$.—Fig. 175. *Spiraea tomentosa* L., var. *alba* West., Metaphase II, $n = 18$.—Fig. 176–180. Rutaceae.—Fig. 176. *Dictamnus albus* L., Metaphase I, $n = 18$.—Fig. 177. *Evodia Daniellii* (Benn.) Hemsl., Metaphase I, $n = 36$.—Fig. 178. *Evodia fraxinifolia* Hook. f., $2n = 72$.—Fig. 179. *Evodia hupehensis* Dode., $2n = 72$.—Fig. 180. *Zanthoxylum Clava-Herculis* L., $2n = ca. 72$.

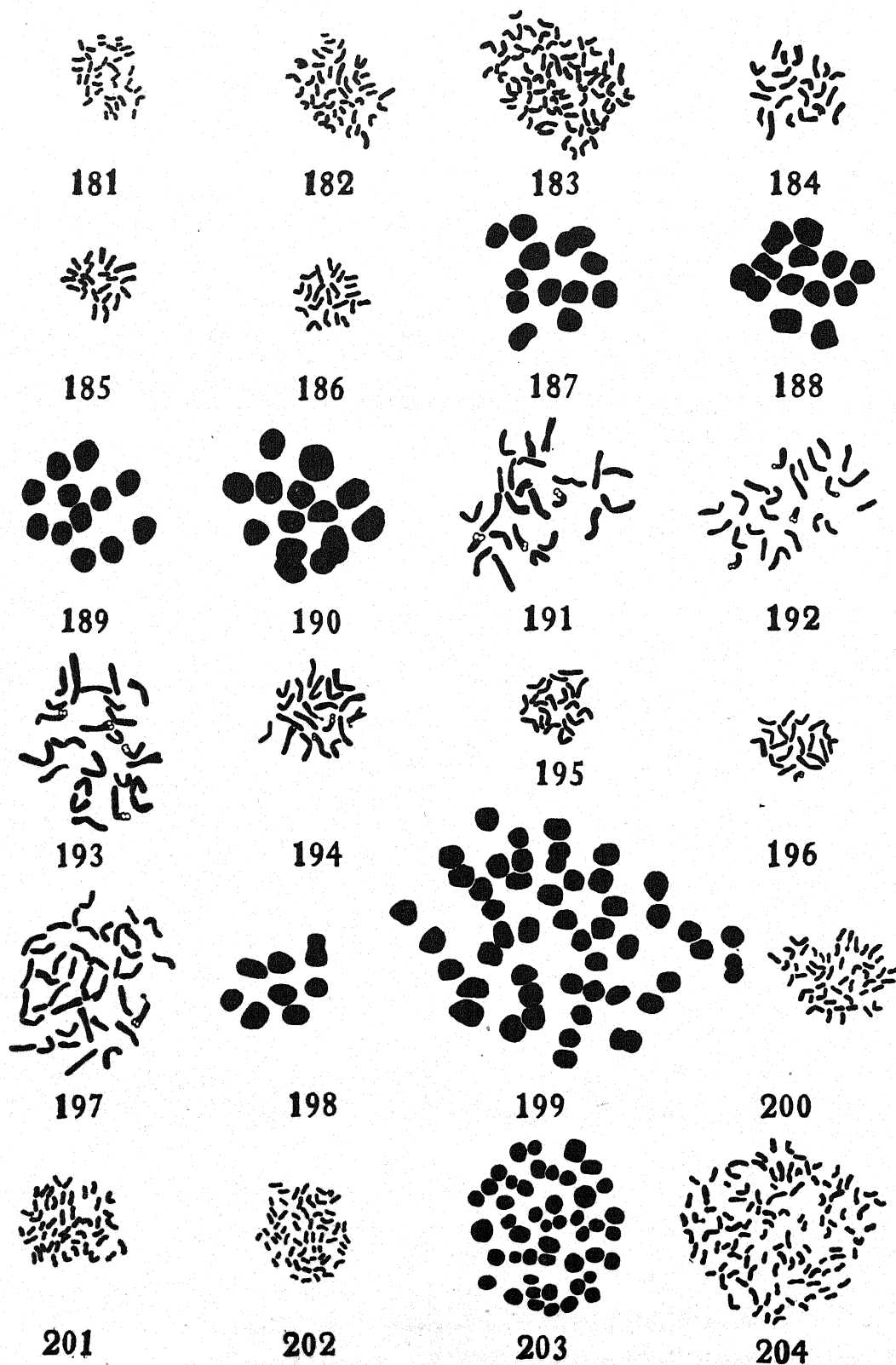


Fig. 181-204.—Fig. 187, 188, 189, 190, 198, 199, 203. Polar views of meiotic stages.—Other figures, polar views of mitotic metaphases.—Magnification $\times 2500$, except figures 187, 188, 189, 190, 198, 199, and 203, which have a magnification $\times 3240$.—Fig. 181, 182, 183. Salicaceae.—Fig. 181. *Salix Humboldtiana* Willd., $2n = 38$.—Fig. 182. *Salix*

mitotic metaphases of root-tip material in the accompanying figures were drawn from the largest cells in the periblem region in the most mitotically-active zone with the exception of figure 76 which was a metaphase in an inner periblem cell. In most cases root tips from small seedlings were inferior to root tips from large well-developed seedlings since cell size was smaller and the chromosomes were not as well spread out on the metaphase plates. These observations emphasize the need for a detailed study of chromosome-size variation in different tissues and organs of individual plants. Several species should be examined but particularly a species such as *Crepis capillaris* which has a distinctive chromosome complement. An examination of individual haploid, diploid, and tetraploid plants would provide conclusive evidence of chromosome-size variation in different tissues and organs of a plant.

Slides of aceto-carmine smears of pollen mother cells usually contained from fifty to several hundred pollen mother cells with metaphase plates. Slides with stained serial sections of root tips had from twenty-five to several hundred metaphase plates per slide. The time required to make an aceto-carmine smear is short, and this method is especially useful when rapid routine chromosome counts are desired, and flowering material is available. Many investigators object to the use of the paraffin-sectioning technique since it is a long tedious process. This investigator has found that, when large numbers of fixations are being uniformly treated, the process becomes routine and many collections can be handled in satisfactory time. The resultant slides with serial sections and abundant metaphase plates with well-stained chromosomes have justified the use of the paraffin-sectioning method. The use of Webber's modification of Nawashin's fixative is advantageous since material can be fixed, washed after twenty-four hours or after a longer time if necessary, and stored in 70 per cent alcohol until required.

Cyto-taxonomic notes.—During this survey numerous examples of cytotaxonomic relationships were observed and the accompanying figures are useful for this purpose. Sometimes cytology can solve or confirm a taxonomic identification. In the Apocynaceae, *Lochnera rosea* (L.) Reichb. proved to be cytologically distinct from four species of the genus *Vinca*. The genus *Incarvillea* was cytologi-

cally distinct from six other genera of the Bignoniaceae. In these two instances, the cytological differences were correlated with differences in plant morphology and habit. In the Cistaceae, plants of *Helianthemum canum* Boiss. had a different chromosome number ($2n = 22$) from the other diploid species of *Helianthemum* ($2n = 20$) which were examined; and plants of *H. canum* Boiss. were morphologically distinct, slower growing, and less vigorous, compared with the other species examined.

Intraspecific chromosome-number variation.—During this investigation diploid and tetraploid races were found in two collections of *Opuntia compressa* Macbride. Steiner (1944) reported $2n = 48$ in two collections of *Talinum teretifolium* Pursh., and $2n = 24$ in one collection; Bowden (present account) found $2n = 48$ in one collection.

Löve and Löve (1942) stated that intraspecific polyploidy was known in only 7 per cent of Scandinavian species. Nawashin (1926) analyzed 4,000 plants of *Crepis tectorum* ($n = 4$) as summarized in table 8, Darlington (1937). Normal individuals numbered 3,957 plants (98.9 per cent of the total); thirty-nine were numerical variants, including five tetraploids; and four were structural variants. Of 2,000 plants of *Crepis capillaris* ($n = 3$), eleven plants were triploid, while 1,989 (99.45 per cent of the total) were normal individuals. Numerous examples of intraspecific polyploidy were listed by Darlington (1937) in table 37.

Granick (1944) listed a series of chromosome numbers determined for plants of *Agave americana*, namely: $2n = 20, 60, 118, 119, 120, 180$, and 226 . Giles (1942) examined fifty-nine field collections of *Cuthbertia graminea* Small, and found that twelve collections were diploid ($2x$), with one triploid ($3x$) plant present in one collection; forty-six collections were tetraploid ($4x$) with one hexaploid ($6x$) and two aneuploids ($4x + 1$) among the collections; and one collection was hexaploid. Baldwin and Culp (1941) demonstrated that there were two different chromosome races of *Diospyros virginiana* L. Seven collections had $2n = 60$ while seventeen collections had $2n = 90$. Baldwin (1941) studied 117 collections of *Galax aphylla*. Eighty collections were diploid, $2n = 12$; thirty-seven collections were tetraploid, $2n = 24$. Of seventy-one collections of *Sedum ternatum* Michx., Baldwin (1942a) reported that eleven collections were diploid ($2n = 16$), fifty-seven collections were tetra-

Bonplandiana H.B. and K., $2n = 42$.—Fig. 183. *Salix babylonica* L., $2n = 76$.—Fig. 184, 185, 186. Sapindaceae.—Fig. 184. *Cardiospermum Halicacabum* L., $2n = 22$.—Fig. 185. *Koeleruteria formosana* Hayata., $2n = 22$.—Fig. 186. *Koeleruteria paniculata* Laxm., $2n = 22$.—Fig. 187–193. Tamaricaceae.—Fig. 187. *Tamarix gallica* L., Metaphase I, $n = 12$.—Fig. 188. *Tamarix hispida* Willd., Metaphase I, $n = 12$.—Fig. 189. *Tamarix odessana* Stev., Metaphase I, $n = 12$.—Fig. 190. *Tamarix parviflora* DC., Metaphase I, $n = 12$.—Fig. 191. *Tamarix parviflora* DC., $2n = 24$.—Fig. 192. *Tamarix pentandra* Pall., $2n = 24$.—Fig. 193. *Tamarix aphylla* (L.) Karst., $2n = 24$.—Fig. 194–197. Ulmaceae.—Fig. 194. *Celtis laevigata* Willd., $2n = 20$.—Fig. 195. *Celtis occidentalis* L., $2n = 20$.—Fig. 196. *Celtis sinensis* Pers., $2n = 20$.—Fig. 197. *Celtis australis* L., $2n = 40$.—Fig. 198, 199. Umbelliferae.—Fig. 198. *Eryngium maritimum* L., Metaphase II, $n = 8$.—Fig. 199. *Eryngium aquaticum* L., Metaphase I, $n = 48$.—Fig. 200–204. Verbenaceae.—Fig. 200. *Clerodendron speciosissimum* Van Beert., $2n = 48$.—Fig. 201. *Clerodendron Thomsoniae* Balfour, var. *delectum* Hort., $2n = ca. 48$.—Fig. 202. *Clerodendron Kaempferi* (Jacq.) Sieb., $2n = 60$.—Fig. 203. *Clerodendron trichotomum* Thunb., Metaphase I, $n = ca. 46$.—Fig. 204. *Clerodendron Bungei* Steud., $2n = 108$.

ploid ($2n = 32$), two collections were triploid ($2n = 24$), and one collection was hexaploid ($2n = 48$). Stebbins and Kodani (1944) have reported much variation in the chromosome numbers of plants in populations of guayule and mariola.

Variation in chromosome number has not been observed in some species which have been studied extensively. Baldwin (1942b) examined specimens of *Oxydendrum arboreum* from thirty-one field stations and all were diploid, $n = 12$ and $2n = 24$.

It is important to consider the possible variability of chromosome number in a population of a species when different chromosome numbers are reported for the same species, rather than to assume that a determination is incorrect.

Differences in chromosome-number reports.—Of the 181 species examined during this survey, the chromosome numbers of 135 species (75 per cent of the total) have not been previously reported by other investigators. Former counts and the present determinations agree for twenty-seven species (59 per cent of the species examined by more than one investigator). In addition there are eight species for which the same chromosome numbers have been observed by the present and one or more previous investigators, but for which there are one or more previous accounts which report different chromosome numbers. The majority report for these eight species confirms the present determinations but does not necessarily mean that the previously reported counts which are not in agreement, are incorrect, since there are many reasons why different chromosome numbers can be found in different individuals of the same species. Thus, the same chromosome numbers have been determined for a total of thirty-five species or 76 per cent of the forty-six species which have been examined by both the present and previous investigators.

Different chromosome numbers reported for species examined by three or more investigators.—The same chromosome number has been reported independently by two or more accounts including the present, for seven species for which one former investigator has reported a different chromosome number. Widely divergent chromosome numbers have been reported by one investigator each for *Manfreda virginica* (L.) Salisb., *Vinca minor* L., *Vinca major* L., *Manihot palmata* (Vell.) Pax., and *Zizyphus Jujuba* Mill. Chromosome numbers which varied by only a small number of chromosomes have been reported for two species, *Campsis grandiflora* (Thunb.) Loisel., and *Hydrophyllum canadense* L. In plants of *Chelidonium majus* L., four investigators, including the present, found $n = 6$; while two reports listed $n = 8$, and $2n = 10$. The validity of the majority reports is supported by chromosome-number data listed by several investigators for related species, e.g., in *Hydrophyllum Vinca*, *Campsis*, and *Manihot*. It is not possible to explain the divergent counts in all cases. Some counts have probably been incorrect; some of the taxonomic identifications may have been incorrect,

or other taxonomic species may have been examined; or individuals may exist in some of these species with different chromosome numbers.

Different chromosome numbers reported by the present account and one previous investigator.—The present paper reports chromosome numbers which differ from those reported by former investigators for eleven species. Widely divergent counts are reported for five species, *Arisaema triphyllum* (L.) Schott., *Helianthemum alpestre* (Jacq.) Dun., *Cordyline australis* Hook. f., *Anthericum Liliago* L., and *Koeleria paniculata* Laxm. Slightly different chromosome numbers are reported for six species, i.e., *Ruellia tuberosa* L., *Periploca graeca* L., *Begonia Evansiana* Andr., and *Incarvillea grandiflora* Bur. and Franch., *Anthericum Liliastrium* L. var. *major* Hort., and *Cocculus trilobus* (Thunb.) DC. The present determinations of these eleven species are supported by cytological data for related species and genera.

Reasons for different chromosome-number reports for the same species.—Assuming that the counts of one investigator are incorrect, any of the following causes may explain different chromosome number reports for a species. (1) The chromosome configurations may have been misinterpreted, as can sometimes be ascertained by an examination of the original drawings, e.g., *Arisaema triphyllum* (L.) Schott. (2) The material may have been poorly fixed or poorly stained so that chromosome structure was difficult to see clearly. (3) In the mitotic metaphases of some species, there are one or more smaller pairs of chromosomes. If these are not observed carefully, a lower chromosome number may be reported. The chromosome complements of *Asimina triloba* (L.) Dunal. (fig. 14) and *Pontederia cordata* L. var. *angustifolia* Torr. (fig. 165) required special attention because of the smaller pairs of chromosomes. (4) In some cases, secondary constrictions and satellites are present and unless these are interpreted correctly, a higher chromosome number may be reported. (5) At metaphase I, univalents are formed in a percentage of the pollen mother cells of certain hybrid material, or in material subjected to unusual environmental conditions such as sudden temperature change, and unless correctly interpreted, a higher chromosome number might be reported. (6) Multivalents are sometimes present at metaphase I; and unless recognized, a lower chromosome number might be listed.

Assuming, on the other hand, that both of the conflicting chromosome number determinations are correct, the following causal factors may be involved in the existence of two chromosome numbers for the same species. (1) The examples of variation of chromosome numbers in a population of a species mentioned earlier emphasize the fact that intraspecific chromosome-number variation may occur. (2) Polyploid species may contain numerous individuals with different chromosome numbers due to the formation by certain polyploid plants of gametes with dif-

ferent chromosome numbers (table 33, Darlington, 1937). (3) The determinations may have been made on materials which belong to two different taxonomic species. It is not always possible to determine from the literature whether the same taxonomic species was investigated by a previous author. The divergent counts noted for *Cordyline australis* Hook. f. and *Helianthemum alpestre* (Jacq.) Dun. are probably both correct, but the plants examined by the different investigators may belong to different taxonomic species. (4) The chromosome numbers of plants in cultivated populations may vary from those in wild populations, since survival value for the progeny may be markedly different. There is often unusual opportunity for inter- and intra-specific hybridization under cultivation, especially in botanical garden collections where groups of species are planted together for demonstration purposes. (5) Under the influence of changes in the environment, both in the field and under cultivation, unusual environmental conditions may cause the formation of gametes with numerical changes in the chromosome complement, and individuals with different chromosome numbers may be developed.

SUMMARY

Cytological examinations were made of 222 collections of 179 species of angiosperms, and two collections of two species of gymnosperms. These were distributed in eighty genera in forty-eight families. Data for each collection included: Blandy Experimental Farm accession number, figure number of drawing of the chromosome complement, taxonomic identification, source of material, chromosome num-

ber determined during this investigation, and chromosome number determined by previous investigators.

The chromosome complements of 177 angiospermous species and one gymnospermous species are figured by 204 camera-lucida drawings. There are 163 drawings of mitotic metaphases of 147 species; and forty-one drawings of meiotic stages (usually metaphase I) of thirty-seven species.

Stained serial sections of root tips are preferred by this investigator for routine studies of the chromosome complement when data on both chromosome number and morphology are required.

Of the 181 species examined during this investigation, the chromosome numbers of 135 species (75 per cent) have not been previously reported by other investigators. Previous chromosome-number reports have been listed for forty-six species.

The present report agrees with that of one or more previous investigators in respect to chromosome number of twenty-seven species. It differs from one previous report but agrees with one or more previous reports for each of seven species; and it differs from two previous reports but agrees with three former accounts in the case of one species. There is majority agreement for thirty-five species or 76 per cent of the forty-six species examined by present and previous investigators. The present list reports a different chromosome number from that listed by one previous report for each of eleven species. The reasons for different chromosome-number reports for the same species are briefly discussed.

McMASTER UNIVERSITY,
HAMILTON, ONTARIO, CANADA

LITERATURE CITED

- BALDWIN, J. T., JR. 1941. *Galax*: The genus and its chromosomes. Jour. Heredity 32: 249-254.
 —. 1942a. Polyploidy in *Sedum ternatum* II. Cytogeography. Amer. Jour. Bot. 29: 283-286.
 —. 1942b. Cytogeography of *Oxydendrum arbo-reum*. Bull. Torrey Bot. Club 69: 134-136.
 —, AND RICHARD CULP. 1941. Polyploidy in *Diospyros virginiana* L. Amer. Jour. Bot. 28: 942-944.
 BOWDEN, WRAY M. 1945. A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae. Amer. Jour. Bot. 32: 81-92.
 CHIARUGI, A. 1925. Embriologia delle Cistaceae. Nuov. Giorn. Bot. Ital. N.S. 32: 223-314.
 COOPER, D. C. 1933. Nuclear divisions in the tapetal cells of certain angiosperms. Amer. Jour. Bot. 20: 358-364.
 DARLINGTON, C. D. 1937. Recent advances in cytology. 2d. ed. Philadelphia.
 ERVIN, CLYDE D. 1941. A study of polysomaty in *Cucumis melo*. Amer. Jour. Bot. 28: 113-124.
 GILES, NORMAN H., JR. 1942. Autopolyploidy and geographical distribution in *Cuthbertia graminea* Small. Amer. Jour. Bot. 29: 637-645.
 GRANICK, ELSA B. 1944. A karyosystematic study of the genus *Agave*. Amer. Jour. Bot. 31: 283-298.
 LÖVE, ÅSKELL, AND DORIS LÖVE. 1942. Chromosome numbers of Scandinavian plant species. Bot. Notiser 1942 (1): 19-59.
 NAWASHIN, M. 1926. Variabilität des Zellkerns bei *Crepis*-Arten in Bezug auf die Artbildung. Zeitschr. f. Zellforsch. u. mikr. Anat. 4: 171-215.
 STEBBINS, G. LEDYARD, JR., AND MASUO KODANI. 1944. Chromosomal variation in guayule and mariola. Jour. Heredity 35: 163-172.
 STEINER, ERICH. 1944. Cytogenetic studies on *Talinum* and *Portulaca*. Bot. Gaz. 105: 374-379.

SOME ADDITIONS TO THE GENUS DODONAEA L. (FAM. SAPINDACEAE) ¹

Earl Edward Sherff

DODONAEA sandwicensis sp. nov.—Nunc frutex 2–3 m. altus nunc arbor circ. 4–6 m. alta, caule usque ad 1.6 dm. crasso; ramis virgatis vel internodiis abbreviatis subflexuoso-tortuosis, inferne teretiusculis superne plus minusve angulatis, sub quoque folio costa elevata notatis, cortice rubrofusco subgriseove glanduloso ceterum glabro. Folia alterna breviter vel moderate petiolata petiolo plerumque marginato et sub 1 cm. longo; lamina elliptica vel oblongo-lanceolata vel subrhomboideolanceolata vel parce oblanceolata spatulatave, apice rotundata obtusave (atque interdum emarginata) vel etiam acuta subacuminatave, basim versus in petiolum angustata, margine plerumque subrepanda et interdum plus minusve revoluta, membranacea vel supra subcoriacea, plerumque 4–8 cm. longa et 1–3 cm. lata (pro surculis sterilibus usque ad 1.5 dm. \times 6 cm.), sicca subpallida ac vix subnitida, nervo mediano valido subtilis prominente convexo, nervis lateralibus subtilibus numerosis parallele oblique patentibus, paucis glandulis adspersa ceterum glabra. Flores dioici (hermaphroditi non visi) in thyrsis terminalibus axillaribusve stipitatis glabris sub 5 cm. longis dispositi, longiuscule pedicellati; sepalis 4–6, plerumque lineari-lanceolatis vel oblongis, glabris vel margine sparsim hispidulis, 1-nervatis, 1–2 mm. longis, usque ad basim plerumque distinctis, in herbario rubro-atris. Florum masculinorum stamina circ. 9, antheris circ. 2 mm. longis filamentis 0.5–0.7 mm. longis vel rarius absentibus; ovario rudimentario superne setuloso; femineorum ovario (sicco) viridi-sucino, viscido ceterum glabrato, nitido, breviter stipitato, 2 stylis (siccis) atro-rubris, glabris, subtilibus, 5–9 (raro –12) mm. longis, apice vel rarissime usque ad basim distinctis, plerumque persistentibus etiam maturo fructu. Fructus subsemper 2-loculatus ac 2-alatus, alis sed non stylis inclusis usque ad 11 \times 11 mm. (rarissime 13 mm. longus et 18 mm. latus), omnino vinoso-ruber colore saepius in herbario persistente, glandulosus et interdum viscosus, aliter glabratus. Semen (normaliter unicum pro loculo) planum vel plano-convexum, ovatum, circ. 3 mm. longum.

Now a shrub 2–3 m. tall, now a tree about 4–6 m. tall, the trunk up to 1.6 dm. thick; branches virgate or with shortened internodes and then somewhat bent and twisted, somewhat terete below but more or less angulate above, marked under each leaf with a raised rib; the bark reddish-brown or grayish, glandular but otherwise glabrous. Leaves alternate, shortly or moderately petiolate, petiole commonly margined and under 1 cm. long; blade elliptic or oblong-lanceolate or subrhomboid-lanceolate or barely oblanceolate or spatulate, at apex rounded or obtuse (and at times emarginate) or even acute or subacuminate, toward base narrowed into a

petiole marginally as a rule subrepand and at times more or less revolute, membranaceous or on upper surface subcoriaceous, somewhat glandular but otherwise glabrous, commonly 4–8 cm. long and 1–3 cm. wide (for sterile shoots up to 1.5 dm. \times 6 cm.), in dry state subpallid and barely subglossy, the strong median nerve salient and convex beneath, the many delicate lateral nerves running parallel and spreading obliquely. Florets dioecious (hermaphroditic not observed), rather long-pedicellate, disposed in terminal or axillary thyrses, these stipitate, glabrate, under 5 cm. long; sepals 4–6, commonly linear-lanceolate or oblong, glabrous or at margin sparsely hispidulous, 1-nerved, 1–2 mm. long, commonly distinct almost to base, reddish-black when dry. Stamens of male florets about 9, their anthers about 2 mm. long, the filaments 0.5–0.7 mm. long or more rarely absent; ovary rudimentary, setulose above. Ovary of female florets greenish-amber when dry, viscid but otherwise glabrate, shining, shortly stipitate; the 2 styles blackish red (when dry), glabrous, delicate, 5–9 (rarely –12) mm. long, at apex or very rarely down to the base distinct, commonly persistent even on the mature fruit. Capsules nearly always 2-loculate and 2-alate, up to 11 \times 11 mm. including the wings but not the styles, or very rarely 13 mm. long and 18 mm. wide, entirely wine-red (the color more often persisting in the herbarium), glandular and at times viscid, otherwise glabrate. Seed (normally a single one to a locule) flat or flat-convex, ovate, about 3 mm. long.

*Specimens examined.*² HAWAIIAN ISLS.—David Douglas 4 (Kew, 2 sheets); Gaudichaud 276 (Voy. sur la Bonite), Aug., 1836 (Par.); William Hillebrand (Gray); Hillebrand & John M. Lydgate (Bish.); Horace Mann & William T. Brigham 719 (Bish.); United States Exploring Expedition under Capt. Wilkes, 1838–1842 (N.Y.; U.S.).

ISL. KAUAI—Otto Degener & Emilio Ordoñez 12,621, open forest, alt. 3,000 ft., Milolii Trail, Kokee, Jan. 3, 1940 (Berl., Brit., Calif., Chi., Del., Gray, Kew, Mo., N.Y., Par., Phila., U.S., etc.); Urbain Faurie 276, Hanapepe, Dec., 1909 (Arn.,

² The following abbreviations are used for the depositories cited in this paper: Arn., Arnold Arboretum, Jamaica Plain; Berl., Berlin Botanical Garden; Bish., Bernice P. Bishop Museum, Honolulu; Brit., British Museum of Natural History, South Kensington; Calif., University of California, Berkeley; Chi., Chicago Natural History Museum (formerly Field Museum of Natural History), Chicago; Corn., Cornell University, Ithaca; Del., Delessert Herbarium, Geneva; Goth., Arboretum (Botanical Garden) of Gothenburg; Gray, Gray Herbarium of Harvard University, Cambridge; Kew, Royal Botanical Gardens, Kew; Minn., University of Minnesota, Minneapolis; Mo., Missouri Botanical Garden, St. Louis; N.Y., New York Botanical Garden; Par., Museum of Natural History, Paris; Phila., Academy of Natural Sciences, Philadelphia; U.S., United States National Museum, Washington, D. C.

¹ Received for publication December 21, 1944.

Par.); *Faurie* 277, alt. 1,000 m., Waimea, Feb., 1910 (Arn., Par.); *Charles N. Forbes* 279-K, Wahiawa Swamp, Aug., 1909 (Bish., Calif.); *F. Raymond Fosberg* 12,745, shrub 3 m. tall, in moist forest, Kanalo Huluhulu Ranger Station, Kokee, Jan. 1, 1936 (Chi., etc.); *Albert S. Hitchcock* 15,542, vicin. of Kahloluamanu, Oct. 26, 1916 (U.S.); *J. August Kusche* 120, high plateau of Waimea, Halemanu to Koholuamanu, 1919 (Arn.); *Miss Marie Neal*, alt. 3,000–3,500 ft., Nualolo Trail, Aug. 14, 1936 (Bish.); *Miss Neal*, alt. 3,500–4,000 ft. Awaawapuhi Trail, Aug. 21, 1936 (Bish.); *Joseph F. Rock* 1,674, Halemanu, Feb. 14–26, 1909 (Gray); *Rock* 1,675, same place and date (Bish., N.Y.); *Rock* 2,178, same place and date (N.Y.); *Rock* 17,106, Kaholuamanu, Oct., 1916 (Bish.); *Harold St. John et alii* 10,713, Na Pali-Kona Forest Reservation, Kokee, Waimea, Dec. 26, 1930 (Bish., Calif.); *idem* 10,998, shrub in thicket along ridge, alt. 1,200 ft., Hanakapiai, Napali coast, Jan. 2, 1931 (Chi.); *St. John & F. R. Fosberg* 13,749, tree 15 ft. tall, in open koa forest, alt. 2,000 ft., Milolii ridge, Waimea, Dec. 27, 1933 (Bish.; Chi.); *St. John & Fosberg* 13,772, shrub 8 ft. tall, in wet forest, alt. 3,200 ft., same place and date (Bish., Chi., etc.); *Carl Skottsberg* 994, near Kokee Station, Waimea, Oct. 28, 1922 (Bish., Goth.); *Truman G. Yuncker* 3,522, alt. 3,600 ft., near Forest Ranger's Station, Kokee, Jan. 20, 1933 (Yunck., U.S.).

ISL. OAHU—*Capt. Beechey* (Kew); *Degener* 11,155, in open woods, Wilhelmina Rise, Honolulu, Feb., 1923 (Chi., Gray); *Degener* 11,167, in rain-forest, Konahuanui above Pauoa Flats, Feb. 25, 1928 (Berl., Calif., Chi., Del., Gray, Kew, Par.); *Degener* 11,183, in wet forest, east slope of Palikea, Oct. 23, 1932 (Berl., Calif., Chi., Gray, Kew, Minn., N.Y., Par., Phila., U.S.); *Degener* 11,188, near summit, Kanehoa, July 5, 1931 (Chi.); *Degener & K. K. Park* 11,179, near forest, southeast side of Makua Valley near its head, Jan. 3, 1932 (Berl., Chi.); *Degener, Park, Takamoto, & Topping* 11,104, in rain-forest, C.C.C. Trail, Aiea, Feb. 16, 1936 (Berl., Chi., Kew, Par.); *Degener & Salucop* 11,194, moderately dry fills, ridge north-northeast of Kaala summit, Apr. 11, 1937 (Arn., Berl., Calif., Del., Gray, Kew, Minn., Mun., N.Y., Par., Phila., U.S.); *Degener, Salucop, & Arlanticco* 11,530, in lower rain-forest, Aiea Ridge, Dec. 6, 1937 (Berl., Brit., Calif., Chi., Del., Gray, Kew, Minn., Mun., N.Y., Par., Phila., U.S., etc.); *C. N. Forbes*, ridge west of Kalihi, Oct. 3, 1908 (Bish., Calif., Mo.); *Forbes*, Palolo Valley ridges, Nov. 7, 1908 (Bish.); *Forbes*, "forest form," slopes of Kaala, Mokuleia, Apr. 26–May 16, 1912 (Bish., Chi., Mo., etc.); *Forbes* (With Dean Lake) 1,975-O, Waimano Ridge, Oct. 27–30, 1914 (Bish.); *Forbes & Rock*, Palolo Valley, Nov. 7, 1908 (Chi., Mo., U.S.); *F. R. Fosberg* 8,960, bush 2.5 m. tall, in wet forest, alt. 690 m., peak at head of Pauoa Flats, Oct. 30, 1932 (Chi., etc.); *Fosberg* 9,108, shrub 3 m. tall, in dry forest, alt. 750 m.,

east slope of Puu Kaala, Waianaeuka, Jan. 8, 1933 (Chi., etc.); *Fosberg* 10,835, slender tree 5 m. tall, in moist forest, alt. 750 m., Puu Kawiwi-Puu Kaala ridge, Makaha-Waianae Kai, Mar. 31, 1935 (Chi.); *D. Wesley Garber* 121, Konahuanui Trail near Pauoa, Jan. 4, 1920 (Bish.); *Garber* 131, Mt. Konahuanui Trail above Pauoa, same date (Bish.); *Charles Guadichaud, Voy. sur la Bonite* (Gray); *William Hillebrand* 144, Nuuanu (Kew); *E. P. Hume* 211, tall shrub, alt. 1,200 ft., on wooded ridge, Puu Peahinaia, Oct. 4, 1931 (Bish.); *Raymond Inafuku*, dry ridge, alt. 1,800 ft., Opaaula Gulch, Paalaa, Nov. 9, 1930 (Bish.); *Noel H. Krauss*, alt. 1,200–1,400 ft., Manoa-Palolo ridge, Nov. 19, 1933 (Bish., Chi.); *Kazuto Nitta* (*O. Degener distrib. no.*) 11,174, alt. 1,500 ft., Kipapa, Nov. 10, 1929 (Chi.); *A. M. Octavio*, Kipapa, Nov. 26, 1929 (Bish.); *Jules Remy* 567, exact locality not stated, 1851–1855 (type, Par.); *Olof H. Selling* 3,656, east side of Waianae Mts., Sept. 25, 1938 (Goth.); *Carl Skottsberg* 266, alt. about 700 m., Palehua etc., Waianae Range, Aug. 25, 1922 (Bish., Goth.); *Skottsberg* 919, Palolo Valley, Koolau Range, Oct. 25, 1922 (Goth.); *Harold St. John* 10,032, shrub, alt. 1,200 ft., on wooded slope, south ridge of Kipapa Gulch, Waipio, Nov. 10, 1929 (Bish., Chi.); *St. John* 10,640, tree 20 ft. tall, on wooded ridge, alt. 1,800 ft., South Opaaula Gulch, Paalaa, Nov. 9, 1930 (Bish.); *St. John* 13,323, tree 20 ft. tall, trunk diam. 6 in., alt. 1,500 ft., in lower woods, ridge south of South Opaaula Gulch, Paalaa, Sept. 24, 1933 (Bish., Chi., etc.); *D. LeRoy Topping* 3,268, Mt. Olympus, Nov. 25, 1925 (Calif., N.Y.).

ISL. MOLOKAI.—*F. R. Fosberg* 13,403, tree 5 m. tall, edge of moist forest, alt. 600 m., Manawai-Kahananui ridge, Dec. 24, 1936 (Chi., etc.); *H. St. John & F. R. Fosberg* 12,866, tree 18 ft. tall, in rain-forest, alt. 2,400 ft., trail on ridge east of Mapulehu Valley, Dec. 29, 1932 (Bish., Chi., etc.).

WEST AND EAST MAUI.—*E. H. Bryan, Jr.*, 653, tree 4 m. tall, trunk diam. 10 cm., in lower forest, alt. 3,000 ft., Haelaau, native name *aalii*, Dec. 21, 1928 (Bish.); *C. N. Forbes* 476-M, Honokahau Drainage Basin, Sept. 25–Oct. 17, 1917 (Bish., Chi., etc.); *Forbes* 481-M, same place and date (Bish., Chi.); *A. S. Hitchcock* 14,802, alt. up to 5,000 ft., Puu Kukui, Sept. 24–26, 1916 (U.S.); *H. Mann & Wm. T. Brigham* 410, West Maui (Chi., Mo.); *Emilio Ordoñez* (*O. Degener distrib. no.*) 12,920, Waihee, Sept. 15, 1940 (Berl., Chi., Del., Kew, N.Y., Par., U.S.); *J. F. Rock* 8,614, tree 15–20 ft. tall, alt. 2,600–3,000 ft., in lower forest of Makawao, Oct. 18, 1910 (Chi., Gray, etc.).

ISL. HAWAII.—*Otto Kuntze* 23,064, alt. 1,400 m., June, 1904 (N.Y.).

This species has been confused with *Dodonaea viscosa* Jacq. In leaf-outline it sometimes faintly suggests var. *vulgaris* f. *repanda* (Schum. & Thonn.) Radlk. of that species, especially if the abnormally large leaves of sterile branches be examined. *D. sandwicensis* is rather easily separated

from the numerous previously described forms of *D. viscosa*, however, in having usually darker, also narrower and more distinct sepals; flowers seemingly always dioecious, not often hermaphroditic; the styles consistently two only, elongate and delicate, and more commonly persistent on the ripe capsules; fruits regularly 2-celled and 2-winged and usually retaining their red or claret color even after drying, not commonly 3-celled and 3-winged and commonly turning stramineous or brownish on drying.

Asa Gray (Bot. U. S. Explor. Exped. 1: 261. 1854) was perhaps the first to publish about this species, although he made no attempt to separate it from *D. viscosa*. He wrote: "... at the latter locality [i.e., Honolulu, Isl. Oahu] also the (probably abnormal) form with elongated styles, some of them 5 or 6 lines in length! Similar specimens were gathered at Oahu by Gaudichaud in the Voyage of the Bonite." The elongate styles are now seen to be entirely normal for this species and to afford a rather striking diagnostic character. In young, numerous flowered, pistillate inflorescences they may even suggest tufts or locks of hair.

In foliage *D. sandwicensis* resembles occasionally *D. viscosa* var. *vulgaris* f. *Burmänniana* (DC.) Radlk. and the latter's counterpart in the Hawaiian flora, described below (see p. 209) as *Dodonaea eriocarpa* var. *glabrescens*.

DODONAEA SANDWICENSIS β *simulans* var. nov.—Folia elliptico-spathulata vel -lanceolata, pallida, apice obtusa vel subacuta, saepius 3–5.5 cm. longa et 1–1.6 cm. lata, venis inferne manifestis. Fructus bialati, parvi, alis inclusis tantum 8–11 mm. lati et 6–8 mm. alti, sicci subbrunnei (forsitan capsula rubido-brunnea), extus interdum obsolete setulosi, stylo brevissimo vel absenti.

Leaves elliptic-spatulate or -lanceolate, pale, apically obtuse or subacute, more often 3–5.5 cm. long and 1–1.6 cm. wide, veins conspicuous beneath. Fruits 2-winged, small, wings included only 8–11 mm. wide and 6–8 mm. tall, brownish when dry (or the capsule perhaps reddish-brown), sometimes obsoletely setulose on surface, style very short or lacking.

Specimens examined: Harold St. John et alii 12,538, alt. 2,200 ft., Maunahui, Kaunakakai, Isl. Molokai, Dec. 24, 1932 (type, Yunck.).

In its veiny leaves and 2-celled capsules and consequently 2-winged fruits, this variety suggests a small-leaved form of *D. sandwicensis* proper. However, its fruits do not exhibit the bright red color characteristic for fruits of *D. sandwicensis*, they are smaller, and they do not have long styles. They suggest in their diminutive size certain forms of *D. viscosa* Jacq. found in Florida of the United States, but are distinctive in the evident constancy of their 2-winged character.³ A few of the 26 fruits

³ One Florida form of *D. viscosa*, separated some years ago as *D. microcarpa* Small (Forreya 25: 39. 1925) has small, 2-winged and 3-winged fruits.

found on the type have a few simple hairs sparsely placed (disregarding certain stellately clustered hyphal branches of some fungus observed in a few small areas), but these hairs will hardly suggest the larger-fruited *D. eriocarpa*.

DODONAEA ERIOCARPA SMITH in Rees Cyclop. 12: *Dodonaea* no. 6. 1809; *DODONAEA ERIOCARPA typica* var. nov.—Shrub, much branched, dioecious or at least in some of the varieties polygamous with staminate, pistillate, and hermaphrodite or perfect flowers on same plant; branchlets pubescent with short, stiff, slender, spreading, mostly simple hairs. Leaves narrowly to widely elliptic-lanceolate or -oblong or -oblanceolate, apically acuminate, basally narrowed into a short (± 1 cm.), supernally narrow-margined, hairy petiole; blade 2–4.5 (or even –8.5) cm. long and usually 8–18 mm. broad, short-hairy (often densely so at the minutely revolute slightly wavy but otherwise entire edges and on veins, particularly underneath), the hairs frequently branched at base as also on the petiole; upper surface of blade at least when dry appearing more or less coriaceous and irregularly viscid-glossy. Pistillate flowers (neither staminate nor hermaphrodite seen for var. *typica*) in panicles at ends of branchlets, pubescent. Sepals 5, narrowly ovate or rarely oblong-elliptic, apically somewhat acute, more or less bristly, later spreading or reflexed, about 1.5–2 mm. long. Pedicel filiform, ± 5 mm. long. Ovary 1 mm. tall and subglobose or presently 2 mm. tall and 4-ridged, very shortly stipitate, densely pubescent with hairs mostly branched at base and more or less stellate. Style slender, 2–5 mm. long, glabrate, twisted, with four unequal, suberect, linear stigmata. Capsule turgid, 5–7 mm. tall, more or less appressed-pubescent and glandular-punctulate, 4- or sometimes 3-winged; the glistening, purplish wings more or less glandular-punctulate, up to 16 mm. long and 5 mm. broad, pubescent on their faces and especially so on their margin, leaving when flattened in pressing a basal sinus ± 2 mm. deep and an apical sinus ± 4 mm. deep. Seeds flattish-ovoid, about 1 mm. across.

Specimens examined: ISL. MOLOKAI.—Joseph F. Rock 6,169, plant about 6 feet tall, in company with *Acacia koa* on dry, open slopes below Mapuloa, half-way between Kamoku and Kaunakakai, southeastern Molokai, Mar. 22, 1910 (Bish., Chi., Gray).

ISL. HAWAII.—Lucy M. Cranwell, O. Selling, & C. Skottsberg 3,204, on lava, along road near Huehue, North Kona, Sept. 11, 1938 (Goth.); Alfred Meebold (Otto Degener distrib. no.) 11,182, Huehue, North Kona (Chi., Deg.); Archibald Menzies, alt. 6,000 feet, "Hawaiian Isls." but doubtless from Hawaii (type collection, Kew); Jules Remy 570, Hawaii, 1851–1855 (Gray, N.Y., Par.); Shea & Stevens, North Kona, Jan., 1928 (Bish.).

The cosmopolitan species *Dodonaea viscosa* Jacq. is so highly polymorphic that it has been suspected

of including the principally Hawaiian *D. eriocarpa* described long ago by Sir James E. Smith. Benth, at the conclusion of an extended study of *Dodonaea* (Fl. Austral. 1: 472. 1863) clearly had *D. eriocarpa* in mind when he mentioned "possibly one distinct Sandwich Island species." However, Hillebrand (Fl. Haw. Isls. 88. 1888) recognized *D. eriocarpa* as valid and retained it. We may note, too, that Radlkofer, in his classical study of *Dodonaea viscosa* Jacq. (Mart. Fl. Brasil. 13^{III}: 643. 1900), accepted a reduction to *D. viscosa*, by Benth, of *D. spatulata* Smith but entirely omitted *D. eriocarpa*, thus impliedly recognizing the latter as valid.

Several years ago I made a study of much Hawaiian material of *Dodonaea* and described, in manuscript, various varieties and forms of *D. eriocarpa*. These have been withheld until a comparative study of many hundreds of specimens of *D. viscosa* from all quarters of the globe could be undertaken. This was recently done, thanks to the aid received in the lending of materials from various institutions. My conclusion that *D. eriocarpa* Smith is a valid species, known only from the Hawaiian Islands (where represented by several subordinate entities as well as by its var. *typica*), from Java, and from the Galapagos Islands is emphatic. It is true that *D. viscosa* exhibits certain forms elsewhere that simulate *D. eriocarpa* in one or another special characters, but the several combinations of characters as shown especially well in varieties or forms of *D. eriocarpa* in the Hawaiian Islands are not found.

Hillebrand (*loc. cit.*) seems to have been unfamiliar with many of these varieties and forms of *D. eriocarpa* and, in the absence of precise knowledge of the type itself, to have treated as mere variants of the species proper the several varieties that he himself had collected.

Fortunately, I have before me a specimen of the original material collected by Menzies, whose collection was cited by Smith in Rees' Cyclopaedia as the type basis for his *D. eriocarpa*. Only the above few specimens cited for var. *typica* match it. Numerous other specimens of *D. eriocarpa* had been labeled to the species in herbaria but these and certain additional ones are seen on further study to fall into fifteen additional, fairly well marked varieties. These are treated below. My foregoing description of the species proper, i.e., var. *typica*, has been drawn in part perforce from the supplementary specimens cited, since the small Menzies branch is inadequately representative. It has ovaries but no mature fruits; and, as remarked originally by Smith himself, no anthers are present. Indeed, on none of the specimens examined for var. *typica* have stamens been observed and it may prove to be, as assumed by Smith, that the plants of var. *typica* are dioecious.

Key to Various Elements of Dodonaea Eriocarpa as Here Presented¹

- a. Leaf-blades often 4-10 (or even -15) cm. long and 2-4.5 cm. broad, hispidulous beneath or at base
 - b. Leaf-blades hispidulous beneath, fruiting clusters dense, often subglobose, plant native to southern Hawaii and perhaps to West Maui
 - var. η *Hosakana*
 - b. Leaf-blades hispidulous at base, fruiting clusters loose and elongate, plant native of Java
 - var. θ *Waitziana*
- a. Leaf-blades shorter or narrower, or if not, then glabrate
 - b. Leaf-blades mostly oblanceolate to obovate or even broadly oblong
 - c. Leaf-blades distally (except for extreme apex) more often obtuse, rounded, or subtruncate, infrequently subacute
 - d. Fruits more often 2-winged; plant native of southeastern Molokai.....var. λ *pallida*
 - d. Fruits mostly 3- or 4-winged; plants not known from Molokai
 - e. Leaves very thin, drying dark purplish-green, fruits large, \pm 2.5 cm. long and wide; plant native of Lanai.....var. θ *oblonga*
 - e. Leaves otherwise, fruits smaller
 - f. Leaves small, blade commonly 2-3.5 cm. long and 1.3-2 cm. wide, fruits small, about 7-10 mm. wide (including wings); plant native of Lanai....var. ξ *lanaiensis*
 - f. Leaves and fruits larger
 - g. Leaves and fruits more or less conspicuously pubescent
 - var. ϵ *obtusior* and f. *galapagensis*
 - g. Leaves and fruits mostly glabrate or weakly pubescent
 - var. ι *Degeneri* and f. *decipiens*
 - c. Leaf-blades distally more often narrowed, from subacute to acuminate
 - d. Fruits large, capsule proper \pm 1.5 cm. long and \pm 1.2 cm. wide; plant native of Hawaii
 - var. γ *Forbesii*
 - d. Fruits smaller or at least with smaller capsules
 - e. Fruits more often 2-winged; plant native of southeastern Molokai
 - var. λ *pallida* f. *acuminatula*
- e. Fruits infrequently 2-winged
 - f. Leaves more often glossy, chiefly somewhat elliptic-lanceolate; ovaries weakly or sparsely hispidulous....var. κ *glabrescens*
 - f. Leaves mostly lacking gloss, chiefly more dilated above middle; ovaries moderately to strongly hispidulous
 - g. Fruits very pubescent; styles very slender and 7-8 mm. long; plant native of Kauai, Oahu, and apparently Lanai
 - var. δ *waimeana*
 - g. Fruits weakly to moderately pubescent, styles shorter and stouter
 - h. Pubescence of ovaries strongly stellate
 - D. eriocarpa* sensu stricto,
 - i.e., var. α *typica*
 - h. Pubescence of ovaries not or but slightly stellate....var. ι *Degeneri* f. *decipiens*
- b. Leaf-blades at most narrowly or a few moderately oblanceolate

¹ Var. π *minor*, described on p. 212, from Java, is as yet too incompletely known to permit inclusion in this key.

- c. Leaves much crowded together, pedicels of pistillate flowers short, thickish, and not or but scarcely visible in the panicle; plant native of northern Hawaii.....var. *β confertior*
- c. Leaves comparatively less congested, pistillate inflorescence more open
- d. Mature fruits small, mostly under 12 mm. tall and (when flattened out) 15 mm. broad including wings
- e. Fruits usually very pubescent even on wings; most leaves strongly acuminate at apex; plant native of southeastern Molokai
var. *ν molokaiensis*
- e. Fruits glabrate; leaves subacuminate to obtuse at apex; plant native of Galapagos Islands.....var. *μ Vaccinioides*
- d. Mature fruits larger
- e. Leaves at most barely acuminate at apex; fruits and ultimate branchlets but weakly pubescent; plant common in East Maui and very rare in northwestern Hawaii
var. *ξ Hillebrandii*
- e. Leaves commonly very acuminate at apex
- f. Fruits very large, capsule proper ± 1.5 cm. long and ± 1.2 cm. wide; leaves glabrate or very slightly pubescent..var. *γ Forbesii*
- f. Fruits smaller
- g. Ovaries slightly hispidulous
var. *κ glabrescens*
- g. Ovaries densely stellate-pubescent
D. eriocarpa sensu stricto,
i.e., var. *α typica*

DODONAEA ERIOCARPA *β confertior* var. nov.—Frutex ± 1.5 m. altus, foliis confertis plerumque oblanceolatis, facile acuminatis, principalium lamina 3–5 cm. longa et saepius 1.2–1.8 cm. lata. Inflorescentiae compactae (floribus confertis ac breviter pedicellatis, tantum femineis visis) quam folia primum et plerumque etiam demum multo breviores. Fructus paulo minores alis usque ad 1.4 cm. longis et aegre vel etiam minime ciliatis, sinu apicali angustissimo.

Shrub ± 1.5 meters tall, the crowded leaves commonly oblanceolate and easily acuminate, blade of principal ones 3–5 cm. long and more often 1.2–1.8 cm. wide. Inflorescences compact (flowers crowded and shortly pedicellate, only the pistillate seen), at first and commonly also at last much shorter than the leaves. Fruits a little smaller, wings up to 1.4 cm. long and weakly or even very slightly ciliate, apical sinus extremely narrow.

Specimens examined: Joseph F. Rock 8,334, shrub 5 feet tall, alt. 6,000 feet, Ahamoia (Ahumoa) Crater, Mauna Kea, Isl. Hawaii, June, 1910 (type, Gray: isotypes, Bish., Chi., Kew).

DODONAEA ERIOCARPA *γ Forbesii* var. nov.—Inflorescentia ramulique moderate patenti-hispidi. Folia oblongo-lanceolata vel pauca angustiora, utrinque (apice longe) acuminata supra fere glabrata infra marginibusque debiliter pubescentia, lamina 4–8 cm. longa et 0.8–2 cm. lata, petiolo gracili circ. 5–10 mm. longo. Fructus (soli maturi sicci rubro-brunnei vel purpurascens visi) magni, nunc 3-saepius 2-alati, alis inclusis ± 2.2 cm. longi et ± 2 cm. lati, sparsim pubescentes, capsula ipsa

maxima ± 1.5 cm. longa et ± 1.2 cm. lata, alis 2–5 mm. latis.

Inflorescence and branchlets moderately spreading-hispid. Leaves oblong-lanceolate or a few narrower, at each end acuminate (at apex elongately so), above almost glabrate, underneath and on margins weakly pubescent, blade 4–8 cm. long and 0.8–2 cm. wide, petiole slender and about 5–10 mm. long. Fruits (only mature, dry, reddish-brown or purplish ones seen) large, now 3- more often 2-winged, ± 2.2 cm. long including wings and ± 2 cm. wide, wings 2–5 mm. wide.

Specimens examined (all from ISL HAWAII): Charles N. Forbes 247-H, Kanehaha, Kona, June 23, 1911 (type, Bish.: isotypes, Calif., Mo., N.Y., Par., U.S.); Horace Mann & William T. Brigham 410, Isl. Hawaii (Gray).

DODONAEA ERIOCARPA *δ waimeana* var. nov.—Folia saepius obovato-oblanceolata, apice acuminata, lamina plerumque 3–4.5 cm. longa et 1.2–1.9 cm. lata. Inflorescentiae femineae juvenes plerumque laxae, stylis gracilioribus et paulo longioribus (circ. 7–8 mm. longis). Fructus pubescentiores.

Leaves more often obovato-oblanceolate at apex acuminate, blade commonly 3–4.5 cm. long and 1.2–1.9 cm. wide. Young pistillate inflorescences commonly lax, styles more slender and a little longer (about 7–8 mm. long). Fruits more pubescent.

Specimens examined: ISL. KAUAI—Abbé Urbain Faurie 275, Waimea, Feb., 1910 (type, Par.); Amos A. Heller 2,846,⁵ on Kaholuamanu, above Waimea, Sept. 30, 1895 (Chi.); Heller (similarly) 2,846, same place, Oct. 1–8, 1895 (Calif., N.Y., U.S.); William Hillebrand, Kauai (Gray); Hillebrand 574, Kauai (Kew); Albert S. Hitchcock 15,559, Kaholuamanu to Waimea, Oct. 27, 1916 (U.S.); Joseph F. Rock 2,646, Kauai (Chi.); Rock 3,992, Kauai (Chi.); Rock 3,996, dry fore hills, Waimea, Feb., 1909 (Gray); Rock 3,997, Kauai (Chi.); Rock 4,000, Kauai (Bish.).

ISL. OAHU—Degener, Martinez, & Salucop 11,080, on moderately dry, open slope, plateau west of Kaumuku Gulch, Puuiki, Mar. 17, 1937 (Arn., Berl., Brit., Calif., Chi., 2 sheets, Del., Goth., Gray, Minn., Mo., Mun., N.Y., Par., Phila., U.S., U.V., etc.; a form with fruits mostly bialate, only very few trialate).

ISL. LANAI—Charles N. Forbes 508-L, Lanai, Sept., 1917 (Bish.; a small, staminate, small-leaved branch apparently belonging with this variety).

DODONAEA ERIOCARPA *ε obtusior* var. nov. et obtusior f. nov.—Folia plus minusve obovata (raro oblonge oblanceolata), supra interdum fere glabra; lamina nunc 2–3.5 cm. longa et ± 1.5 cm. lata nunc ad 6 cm. longa et ad 3 cm. lata, apice obtuso vel saepius rotundo subtruncato saepe abrupte mucronata vel apiculata; sepalis nunc ovatis nunc lanceolatis vel linearibus.

⁵ Elsewhere (Minn. Bot. Studs. 1: 849. 1897), Heller says of this plant: "On the bare slopes between the forks of the Waimea River, Kauai, is a very common plant . . . bushes vary in size from two to four feet, with ascending branches."

Leaves more or less obovate (rarely oblong-oblancoate), at times almost glabrous above; blade now 2–3.5 cm. long and ± 1.5 cm. wide now up to 6 cm. long and to 3 cm. wide, often abruptly mucronate or apiculate at the obtuse or more frequently rounded or subtruncate tip; sepals now ovate now lanceolate or even linear.

Specimens examined: ISL. NIIHAU—J. F. G. Stokes, at foot of mountain on west side, Jan., 1912 (Arn., Bail., Bish., Calif., N.Y., U.S., 2 sheets).

ISL. KAUAI—Otto Degener 11,157 (see also under Isl. Hawaii), Kaulaula Ridge, dry region, June 13, 1926 (Berl., Chi., Deg.); Degener & Emilio Ordoñez 12,620, scrub at alt. 2,500 feet, Puehu Ridge, Waimea, Dec. 29, 1939 (Arn., Berl., Brit., Chi., Del., Goth., Gray, N.Y.); Charles N. Forbes, Waimea, Sept., 1909 (type, Bish.); Forbes 279-K, Wahiawa Swamp, Aug., 1909 (Bish., where one of three specimens approaches and Mo., where one of two specimens approaches or apparently is the var. *Waimeana*; U.S.); Horace Mann & William T. Brigham 410-a, alt. 1,500 ft., Waimea (Del., Mo., U.S.).

ISL. HAWAII—Otto Degener 11,157 (see also under Isl. Kauai), in dry region, 19 miles along main road from Waimea toward Kona, Aug. 18, 1926 (Berl., Chi., Deg.); Mrs. Violet Oliveira Fosberg 46, dry pasture lands, below Kamuela, South Kohala, Aug. 8, 1936 (Bish.); United States Exploring Expedition under Capt. Wilkes, Hawaii, 1838–1842 (U.S.).

The specimens by Mann & Brigham and by the U. S. Exploring Expedition have leaves less rounded at the tip than in the other material examined, but otherwise seem inseparable from var. *obtusior*. Forbes, under his no. 279-K, seems to have confused specimens of this variety with some of var. *waimeana*.

DODONAEA ERIOCARPA var. ϵ *obtusior galapagensis* f. nov.—Folia saepius obovata apice rotundata vel subtruncata (mucrone brevi), nervis lateralibus magis conspicuis, marginibus saepe irregulariter revolutis.

Leaves more often obovate and apically rounded or subtruncate (mucro short), lateral nerves more conspicuous, edges often irregularly revolute.

Specimens examined (all from ALBEMARLE ISL., GALAPAGOS ISLANDS): R. E. Snodgrass & E. Heller 244, alt. 3,000 feet, mountain east of Tagus Cove, Feb. 1, 1899 (Gray); Snodgrass & Heller 876, alt. 4,000 feet, Tagus Cove, June 15, 1899 (Gray, 2 sheets); Snodgrass & Heller, 904, alt. 2,100 feet or less, Tagus Cove, same date (Gray, 2 sheets); Alban Stewart 1,945, bushes 4–5 feet tall, abundant on lava beds above 2,000 feet, Tagus Cove, Mar. 27, 1906 (type, Gray: isotype, N.Y.); Stewart 1,946, small trees and bushes, alt. around 1,800 feet, Cowley Bay, Aug. 10, 1905–1906 (Gray).

The Galapagos specimens here cited are close to the typical form (i.e., forma *obtusior*) of var. *obtusior* except as noted. Phytogeographically, the

occurrence in the Galapagos Islands of this forma and the var. *Vaccinioides* (see below) of an otherwise principally Hawaiian Island species is interesting. Robinson (Proc. Amer. Acad. Arts & Sci. 38: 4: 214. 1902) has already noted "on the whole rather slight traces of affinity between the flora of the Galapagos Islands and that of the Sandwich [i.e., Hawaiian] Islands."

DODONAEA ERIOCARPA ζ *lanaiensis* var. nov.—Folia principalia obovata vel late elliptico-oblonga, minora, lamina sub 4 cm. longa et sub 2 cm. lata. Fructus parvi, plerumque 3- interdum 2-alati, alis inclusis plerumque 7–10 mm. lati, moderate hispiduli.

Principal leaves obovate or widely elliptic-oblong, smaller, blade under 4 cm. long and under 2 cm. wide. Fruits small, commonly 3- sometimes 2-winged, commonly 7–10 mm. wide including wings, moderately hispidulous.

Specimens examined (all from ISL. LANAI): F. Raymond Fosberg 12,520, low, spreading bush, on eroded slope, alt. 500 m., flats above head of Hawaulanui Gulch, Dec. 1, 1935 (type, Chi.: isotypes, several but undistributed as yet); William Hillebrand, Isl. Lanai (Gray); George C. Munro, Isl. Lanai (Bish.).

In the type collection the fruits are 2- and 3-alate, and under 1 cm. long and under 1.2 cm. wide including wings.

DODONAEA ERIOCARPA η *Hosakana* var. nov.—Demum arbor fruticosa ± 2.4 m. alta. Folia nunc lanceolato-oblonga nunc oblanceolata nunc anguste obovata, majora, lamina saepe 4–10 (etiam –12) cm. longa et 2–4.5 cm. lata, apice nunc rotundata vel etiam subtruncata ac abrupte apiculata (pro typo) nunc obtusa vel etiam subacuminata; venis principalibus subtus saepe elevatis magis conspicuis. Paniculae multiflorae ad anthesin apertae et foliis longe superatae. Sepala minora sub 1.5 mm. longa, pro floribus masculinis plerumque angustiora etiam oblongo-lineararia; antheris circ. 1.8 mm. longis, sparsim setulosis; pedicello gracillimo saepe ± 1 cm. longo.

Finally a shrubby tree ± 2.4 meters tall. Leaves now lanceolate-oblong now oblanceolate now narrowly obovate, larger, blade often 4–10 (even –12) cm. long and 2–4.5 cm. wide, at tip now rounded or even subtruncate and abruptly apiculate (for the type) now obtuse or even subacuminate; principal veins often salient beneath and more conspicuous. Panicles many-flowered, at anthesis open and much surpassed by the leaves. Sepals smaller, under 1.5 mm. long, for the staminate flowers commonly narrower even oblong-linear; anthers about 1.8 mm. long, sparsely setulose; pedicel very delicate, often ± 1 cm. long.

Specimens examined (all from ISL. HAWAII):⁶ Otto Degener 11,159, in rocky pasture, Punaluu, July 25, 1926 (Berl., Chi., Deg.); Degener 11,160,

⁶ There seems some possibility that var. *Hosakana* occurs in at least one locality in West Maui. Degener, Ordoñez, & Salucop 12,490 is this variety and was said to

bushy tree about 8 feet tall, in *aa* lava desert, near Hanuapo, July 22, 1926 (Berl., Chi., Deg.); *Degener* 11,161, in *aa* lava flow, Isl. Hawaii, July 22, 1926 (Deg.); *Degener* 11,162, in Kau Desert, 25 miles along main road from Kilauea, July 22, 1926 (Berl., Chi., Deg.); *Degener* 11,171, not common, in arid region, *aa* lava kipuka two-thirds way from Waiohinu toward Kaalualu, Sept. 13, 1929 (Berl., Chi., Deg.); *Degener* 11,173, alt. 400 feet, in dry, rocky region, Milolii, Sept. 20, 1929 (Berl., Chi., Deg.); *Degener* 11,175, dry *aa* lava flow, Punaluu, Mar. 17, 1930 (Berl., Chi., Deg.); *Degener* 11,176, dry *aa* lava kipuka, between Honuapo and Hilea, Feb. 17, 1930 (Berl., Chi., Deg.); *Degener*, *Yasuma Iwasaki*, & *Yoshimasa Nitta* 3,906, "ripe capsules yellowish, never red as at Milolii," arid *aa* lava flow, between Hilea and Honuapo, Apr. 13, 1930 (Berl., Chi., Deg.); *Degener* & *Y. Nitta* 3,966, arid *aa* lava flow, between Hilea and Honuapo, May 7, 1930 (Berl., Chi., Deg.); *F. Raymond Fosberg* 10,143, bush 3 meters tall, on *aa* lava, alt. 230 meters, Volcano Road below Pahala, Aug. 31, 1933 (Chi., etc.); *Miss Marie C. Neal*, tree 8 feet tall, on *aa* flow, alt. \pm 300 feet, Punaluu, Kau, Aug. 8, 1929 (Chi.); *Clifford Nishina* (*Degener distrib. no.*) 11,185, Kona District (Arn., Chi.); *Joseph F. Rock* & *W. M. Giffard*, Kau and Kilauea, Apr., 1911 (Bish.); *Rock* & *Giffard* 8,781, alt. 1,000 feet, lava fields, Hilea, Kau, Apr., 1911 (type, Gray, a staminate and a pistillate branch on same sheet: isotypes, Bish., 3 sheets, Calif., Gray, N.Y., U.S.); *Rock* & *Giffard* (similarly) 8,781, Hilea, July, 1911 (Arn., Gray).

A variety usually recognizable at a glance by its large leaves. From notes accompanying herbarium specimens from the Bishop Museum, it is seen that some preliminary work had once been done toward segregating this variety by Mr. Edward Yataro Hosaka of that institution, and the variety thus may be named very appropriately in his honor.

DODONAEA ERIOCARPA θ **oblonga** var. nov.—Ovariis exceptis fere omnino glabrescens. Folia plerumque oblonga interdum obovata, apice obtusa vel subacuta, basi in petiolum brevum (\pm 5 mm.) cuneate angustata, membranaceissima, lamina \pm 7 cm. longa et \pm 3 cm. lata, sicca plus minusve atropurpureo-viridia. Flores masculini non visi. Styli breves (1–6 mm.), nunc cohaerentes nunc paene distincti. Fructus magni, demum 3 vel 4 alis inclusis \pm 2.5 cm. longi latique.

Almost entirely glabrescent except for ovaries. Leaves commonly oblong but sometimes obovate, apically obtuse or subacute, basally cuneate-narrowed into a short petiole (\pm 5 mm.), very thin; have come from northwest of Hopoi Camp, West Maui, July 28, 1939. It should be noted, however, that at the same place and on the same date the same collectors obtained a large suite of the very different var. *Degeneri*. Because of this fact, I have suspected an admixture of plants from Hawaii (var. *Hosakana*) among plants from West Maui (var. *Degeneri*) during the process of writing labels. In any event, the definite occurrence of var. *Hosakana* in West Maui is in need of confirmation.

blade \pm 7 cm. long and \pm 3 cm. wide, more or less dark purplish-green when dry. Staminate flowers not seen. Styles short (1–6 mm.), now cohering now almost distinct. Fruits large, finally \pm 2.5 cm. long and wide including the 3 or 4 wings.

Specimens examined (all from LANAI): *Charles N. Forbes* 18-L, mountains near Koele, June, 1913 (topotypes, Bish., Mo.); *Forbes* 90-L, same place and date (type, Bish.: isotypes, Calif., Mo., N.Y.); *Joseph F. Rock* 8,049, on open forehills of Mahana, July 27, 1910 (Chi., Gray).

DODONAEA ERIOCARPA ι **Degeneri** var. nov. et **Degeneri** f. nov.—Folia parva, moderate vel late oblanceolata, membranacea, marginibus venisque hispidula alibi glabrata, supra saltem primum valdus viscida, lamina saepius 3–4 cm. longa et 0.8–1.4 cm. lata, apice plerumque acuta vel parce acuminata. Fructus numerosi sed laxe dispositi, plerumque multo minores, conspicue turgidi, omnino sparsim hispiduli, alis parvis saepius sub 7 mm. longis et 2 mm. latis.

Leaves small, moderately or broadly oblanceolate, thinner, on edges and veins hispidulous elsewhere glabrate, above more viscid at least at first; blade more often 3–4 cm. long and 0.8–1.4 cm. wide, at apex commonly acute or barely acuminate. Fruits numerous but loosely disposed, commonly much smaller, conspicuously turgid, sparsely hispidulous all over, the small wings more often under 7 mm. long and 2 mm. wide.

Specimens examined (all from WEST MAUI): *Otto Degener* 11,163, in arid region, Pohakea Gulch, July 11, 1927 (Berl., Chi., Deg.); *Degener*, *Emilio Ordoñez*, & *Felix Salucop* 12,488, extremely arid forehill, Ukumehame Gulch, Aug. 25, 1939 (Arn., Berl., Calif., Chi., Deg., Gray, Kew, Par.); *Degener*, *Ordoñez*, & *Salucop* 12,491, alt. 1,000 feet, on dryish, grassy ridge, Puu Lio northwest of Hopoi Camp, July 28, 1937 (type, Chi.: isotypes, Arn., Berl., Calif., Chi., Deg., Del., Flor., Gray, Kew, Minn., Mo., Mun., Mus.V., N.Y., Par., Phila., U.S., U.V., etc.); *Charles N. Forbes* 120-M, Hanaula, June, 1910 (Bail., Bish., Calif., Mo., N.Y., U.S.; a form approaching in larger size of fruit on certain specimens, but not otherwise, the var. *typica*); *Yoshimasa Nitta* (*Otto Degener distrib. no.*) 11,202, alt. about 1,200 feet, two gulches southeast of Ukumehame Gulch, May 10, 1937 (Berl., Chi., Deg.).

Named after Mr. Otto Degener, who very generously placed at my disposal his entire collections of the Hawaiian species of *Dodonaea*, and whose numerous specimens of this variety (most of them as yet undistributed to herbaria) have proved of the utmost help in drawing the description.

In well developed specimens, the leaves are thinner in texture, farther apart, and somewhat broader than in the var. *Hillebrandii* of East Maui.

DODONAEA ERIOCARPA ι **Degeneri** **decipiens** f. nov.—Folia plerumque \pm dimidio majora, apice nunc rotunda nunc acuta vel conspicue acuminata, saepe longe graciliterque petiolata petiolo 1–3 cm.

longo. Fructus (quam pro f. *Degeneri*) plerumque majores.

Leaves commonly larger by a half more or less, at tip now rounded now acute or conspicuously acuminate, often elongately and slenderly petiolate, petiole 1–3 cm. long. Fruits commonly larger (than for f. *Degeneri*).

Specimens examined: ISL. KAUAI—*Degener & Ordoñez* 12,619, dryish valley side, alt. 500 feet, Hanapepe, Dec. 29, 1939 (Arn., Berl., Brit., Calif., Chi., Del., Flor., Gray, Kew, Minn., Mo., Mun., Mus.V., Par., Phila., U.S., U.V., etc.); *Jared G. Smith*, Mana, Mar. 10, 1908 (Mo.).

ISL. OAHU—*Degener* 11,177, not at all glutinous to touch, on arid slope, Koko Crater, Apr. 2, 1931 (Arn., Berl., Calif., Chi., Corn., Par.); *Degener & K. K. Park* 11,178, on hills east of Kawaihapai, dry, rocky region near sea level, Apr. 12, 1931 (Arn., Berl., Carn., Chi., Corn., Del., Gray, Kew, Minn., Mo., Mun., Mus.V., N.Y., Par., Phila., U.S., U.V., etc.); *Degener & K. K. Park* 11,187, on hill east of Kawaihapai, Apr. 11, 1931 (Chi.); *Degener & M. Takamoto* 11,103, dry, grassy region, middle Palawai Ridge, May 12, 1936 (Arn., Brit., Calif., Chi., Del., Gray, Kew, Minn., Mo., Mun., N.Y., Par., Phila., U.S., U.V.); *Degener, Topping, Martinez, & Salucop* 11,195, below forest, ridge directly north of Kaala summit, Mar. 26, 1937 (Arn., Berl., Brit., Calif., Chi., Gray, Kew, U.S.); *Degener, Topping, Whitney, & Martinez* 11,072, on grassy slope, Waimea Valley, Mar. 6, 1939 (Arn., Berl., Brit., Calif., Chi., 2 sheets; Del., Goth., Gray, Kew, Len., Minn., Mo., Mun., Mus.V., N.Y., Par., Phila., U.S., U.V.); *Charles N. Forbes (with Dean Lake)* 2,278-O, talus slopes near Kaena Point, Dec. 16, 1915 (Bish.); *Forbes* 2,506-O, Wailupe Valley, May 4, 1917 (type, Bish.); *F. Raymond Fosberg* 13,157, shrub 1.5 meters tall, on dry rocky slope, alt. 150 meters, on bluff above Kaena Point, May 31, 1936 (Chi., etc.); *F. R. Fosberg* 13,881, shrub 1 meter tall, alt. 100 meters, southwest side, on dry, steep slope, Diamond Head Crater, May 28, 1937 (Chi., etc.); *F. R. & V. O. Fosberg* 92, bush 2.5 meters tall, alt. 90 meters, on dry, bushy slope, east slope of Kalama Valley, Maunaloa, Mar. 7, 1937 (Chi., etc.); *Mrs. V. O. Fosberg* 91, bush 2 meters tall, on dry, bushy slope, east slope of Kalama Valley, Mar. 7, 1937 (Chi., etc.); *Mrs. Fosberg & J. M. Oliveira* 97, on dry brushy slope, alt. 150 meters, east slope of head of Kalama Valley, Mar. 7, 1937 (Chi.); *E. Y. Hosaka* 776, alt. 600 feet, on dry slope, south ridge, Kipapa Gulch, Waipio, Sept. 25, 1932 (Bish.); *E. P. Hume* 166, on open slope, alt. 450 meters, north ridge, Kaaawa, Apr. 12, 1931 (Bish., N.Y.; foliage faintly suggestive of *D. viscosa* var. *vulgaris* f. *repanda* (S. & T.) Radlk.); *V. Mac Caghey*, Keawaula, Kaena, Mar. 28, 1915 (native name, *a-alii*, Bish., Par., U.S.); *Jules Remy*, 569, Isl. Oahu, 1851–1855 (Par.); *D. LeRoy Topping* 2,821, Makua Valley, Aug. 24, 1924 (Arn., Chi.);

G. P. Wilder, Schofield Barracks, Feb. 14, 1934 (Bish.).

ISL. LANAI—*Albert S. Hitchcock* 14,687, on open lower plain, Sept. 21, 1916 (U.S.); *Emilio Ordoñez (O. Degener distrib. no.)* 12,846, July 14, 1940 (Arn., Berl., Calif., Chi., Del., Gray, Kew, Par.); *George C. Munro*, Isl. Lanai (Bish.).

ISL. MAUI—*Otto Degener* 11,164, north mauka of Ulupalakua, July 4, 1927 (Arn., Berl., Chi., Del., Gray, Kew, Par.); *C. N. Forbes* 1,808-M, Kanaio, East Maui, Mar. 2, 1920 (Bish., Chi., etc.); *Forbes* 2,009, Auwahi, south slope of Haleakala, East Maui, Mar. 18, 1920 (Bish., Chi., etc.); *Gerrit P. Wilder*, Isl. Maui, 1913 (Bish.).

ISL. HAWAII—*C. N. Forbes* 977-H, on kipukas in lava flow of 1823, at level of Kaewewai, June 28, 1915 (Bish.).

HAWAIIAN ISLS.—*Horace Mann & William T. Brigham* 532 (Bish.).

Through the forma *decipiens* the var. *Degeneri* approaches, at times closely, the more obovate-leaved forms of var. *glabrescens*.

DODONAEA ERIOCARPA κ **glabrescens** var. nov.—Folia membranacea, subnitida vel nitida, elliptico-lanceolata vel -oblanceolata, apice plus minusve elongate acuminata, margine paulum repanda, lamina plus minusve glabrescentia, saepius 6–10 cm. longa et 1.6–2.5 cm. lata, sicca brunnescenti-viridia costa mediana inferne straminea. Sepala ovata vel superne angustata. Ovaria debiliter hispidula, fructibus inflorescentiae ramulisque glabrescentibus.

Leaves membranaceous, subglossy or glossy, elliptic-lanceolate or -oblanceolate, at tip more or less elongately acuminate, at margin slightly repand; blade more or less glabrescent, more often 6–10 cm. long and 1.6–2.5 cm. wide, brownish-green (and the median rib straw-colored beneath) when dry. Sepals ovate or upwardly narrowed. Ovaries weakly hispidulous, fruits and branchlets of the inflorescence glabrescent.

Specimens examined: ISL. KAUAI—*Amos A. Heller* 2,871, on Kaholuamanu, above Waimea, Sept. 10–16, 1895 (Bish., N.Y.); *Heller* (similarly) 2,871, same place, Oct. 1–8, 1895 (Chi.); *Heller* (similarly) 2,871, same place, Oct. 11–16, 1895 (Arn., Calif., Mo., N.Y., Par., 2 sheets, Phila., U.S.).

ISL. OAHU—*Otto Degener* 12,390, on dryish forehill with *Santalum*, east slope of Kanehoa, June 17, 1939 (Arn., Berl., Brit., Calif., Chi., Corn., Del., Gray, Kew, Minn., Mo., Mun., Mus.V., N.Y., Par., Phila., U.S., U.V., etc.); *Degener, Ordoñez, & Foster* 12,340, in forest, Honouliuli Trail on southeast slope of Puu Hapapa, May 21, 1939 (Berl., Calif., Chi., Del., Gray, Kew, Minn., Mo., Mun., Par.); *Degener & Park* 11,180, southeast side of Makua Valley near its head, Jan. 3, 1932 (Berl., Calif., Chi., Corn., Del., Minn., Par., U.S.); *Degener & Salucop* 11,194, moderately dry forehills, ridge north northeast of Kaala summit, Apr. 11, 1937 (Berl., Calif., Chi., Del., Gray, Kew,

Mo., Mun., N.Y., Par., Phila., U.S., etc.); *F. Raymond Fosberg & Katherine Duker* 9,049, small tree 2.5 meters tall, in dry forest, alt. 480 meters, head of Makua Valley, Waianae Mts., Makua, Nov. 25, 1932 (type, Chi.); *Fosberg & Duker* 9,051, bush 2.5 meters tall, dry, bushy forest, alt. 475 meters, head of Makua Valley, same date (Chi.); *Olof H. Selling*, cult. in Kamehameha School garden, Honolulu, June 30, 1938 (Goth.); *St. John & Fosberg* 12,179, shrubs 10 feet tall, alt. 1,800 feet, east ridge of 2nd gulch east of Puu Kaupakuhale, northeast slope of Puu Kaala, Mokuleia, Oct. 23, 1932 (Chi.); *D. LeRoy Topping* 2,821, Makua Valley, Aug., 1924 (Calif.); *Topping* 3,166, Makua Valley, May 30, 1925 (Calif., Chi.); *Yunker & Hosaka* 3,208, alt. 2,500 feet, gulch east of Puu Kaupakuhale, Mokuleia, Kamananui, Puu Kaala, Oct. 23, 1932 (U.S., Yunc.).

Heller (Minn. Bot. Studs. 1: 849. 1897) wrote: "This particular form . . . is a tree fifteen feet high, with slender, wide-spreading branches. The leaves are two to four inches long, elliptical-lanceolate, on short petioles, thin, shining, and with margins somewhat undulate. The young leaves are viscid." Heller referred his Kauai specimens hesitantly to *D. viscosa*, but the considerable proportion of 4-alate fruits and the stellate pubescence of many of the fruits even in old age (e.g., *Fosberg & Duker* 9,051) on the Oahu specimens place the variety with *D. eriocarpa*. All of the Kauai specimens examined were staminate, but appeared identical in foliage with some of the Oahu specimens.⁷

DODONAEA ERIOCARPA λ **pallida** Degener & Sherff, var. nov. et f. **pallida** Deg. & Sherff f. nov. —Varietati *molokaiensis* affinis. Folia pallida, spathulata vel obovata, apice plus minusve rotundata et saepe mucronata (raro subtruncata etiam emarginata), basim versus in petiolum gracilem \pm 1.5 cm. longum angustata, glabrata, membranacea, lamina principalia 4–6.5 cm. longa et 1.3–2.7 cm. lata. Inflorescentia feminea subglabrescens, ferax, sepalis saepius lanceolatis, ovario plus minusve albidosetoso, stylo subintegro sub 5 mm. longo. Fructus fere glabrati, interdum 3-saepius 2-alata, alis magnis inclusis \pm 1.5 cm. lata et \pm 1 cm. alta.

Allied to var. *molokaiensis*. Leaves pale, spatulate or obovate, at apex more or less rounded and often mucronate (rarely subtruncate or even emarginate), toward base narrowed into a slender petiole \pm 1.5 cm. long, glabrate, membranaceous, principal ones with a blade 4–6.5 cm. long and 1.3–2.7 cm. wide. Pistillate inflorescence glabrate, prolific, sepals more often lanceolate, ovary more or less whitish-setose, style subentire and under 5 mm. long. Fruits almost glabrate, at times 3- more often 2-winged, the large wings included \pm 1.5 cm. wide and \pm 1 cm. tall.

Specimens examined (all from SOUTHEASTERN MOLOKAI): *Otto Degener* 11,166 *pro*

⁷ We may note, however, that the bialate-fruited *D. sandwicensis* has been collected in the same locality where Heller collected (see *Hitchcock* 15,542, p. 203).

parte, on arid, rocky slope, east part of Kaluaaha plateau, July 3, 1928 (type, Arn.: isotypes, Berl., Calif., Chi., Del., Kew, Minn., Mo., Mus.V., N.Y.).

The description has been based upon the large assortment of pistillate specimens examined. Staminate and monoecious and additional pistillate specimens were collected under the same number at the same place and date, but these have smaller and mostly acuminate or at least subacuminate leaves. They may be set off as:

DODONAEA ERIOCARPA var. λ **PALLIDA** f. **acuminatula** Deg. & Sherff, f. nov.—Folia paulo minora, apice plerumque acuta subacutave plus minusve acuminata.

Leaves a little smaller, at the commonly acute or subacute apex more or less acuminate.

Specimens examined (SOUTHEASTERN MOLOKAI): *Degener* 11,166 *pro parte*, on arid, rocky slope, east part of Kaluaaha plateau, July 3, 1928 (type, almost purely staminate, Chi.: pistillate isotype, Chi.; almost purely staminate isotypes, Brit., Carn., Corn., Flor., Gray, Kew, N.Y., Par., U.S.).

Hillebrand (Fl. Haw. Isls. 88. 1888) noted for *Dodonaea eriocarpa* that the plants were "polygamous with male, female and hermaphrodite flowers (the latter rare) on the same plant." On the labels for his no. 11,166, Degener had written: "dioecious but staminate plants occasionally with fertile flowers." Under the no. 11,166, Degener had sent me for study numerous specimens, these at once recognizable as being of three kinds. The first kind was exclusively pistillate and had broadly tipped leaves; it became the basis for var. *pallida* described above. The second kind, consisting of several branches, had nearly all the numerous flowers staminate, but here and there had been a pistillate flower that more or less completely matured a lone fruit; the leaves were somewhat smaller and more or less acuminate at the apex. This second kind, as also the third, similar to the second in leaves but exclusively pistillate, became the basis for f. *acuminatula*.

Through f. *acuminatula*, var. *pallida* approaches somewhat var. *molokaiensis* of the same region in Molokai, but that variety has considerably smaller and more remote leaves, also smaller and mostly more pubescent fruits.

DODONAEA ERIOCARPA μ **Vaccinioides** var. nov. —Folia glabrata vel glaberrima, petiolo gracillimo saepius 3–10 mm. longo, lamina 1.5–6 cm. longa et 0.5–2 cm. lata, nunc lineari-elliptica nunc oblanceolata vel etiam oblanceolato-oblonga, apice obtusa vel subacuminata et minute mucronata, margine integra vel obscure repanda. Inflorescentiae parvae et (saltem masculinae) numerosae, ramulis pedicellisque subtilibus debilibus pubescentibus; floribus fertilibus plerumque plus minusve hermaphroditis, sepalis pubescentibus oblongis vel oblongo-lanceolatis demum reflexis, stylo gracili circ. 4–5 mm. longo; antheris persistentibus; floribus masculinis sterilibusque saepe pendulis, sepalis ovatis ciliolatis sed dorso subglabris. Fructus raro

2- plerumque 3-alati, sub 1.1 cm. alti et 1.7 cm. lati, glabrati.

Leaves glabrate or very glabrous, the petiole very delicate and more often 3-10 mm. long; blade 1.5-6 cm. long and 0.5-2 cm. wide, now linear-elliptic now oblanceolate or even oblanceolate-oblong, at apex obtuse or subacuminate and minutely mucronate, the margin entire or obscurely repand. Inflorescences small and (at least the staminate) numerous, branchlets and pedicels slender and weakly pubescent; fertile flowers commonly more or less bisexual, their oblong or oblong-lanceolate sepals pubescent and finally reflexed, style slender and about 4-5 mm. long; anthers persistent; staminate and sterile flowers often pendulous, their sepals ovate and ciliate but dorsally glabrate. Fruits rarely 2- commonly 3-winged, under 1.1 cm. tall and 1.7 cm. wide, glabrate.

Specimens examined (all from GALAPAGOS ISLANDS): *G. Baur* 62, Cowley Bay, Albemarle Isl., Aug., 1891 (Gray); *Baur* 63, southern portion of Albemarle Isl., July, 1891 (Gray); *Alban Stewart* 1,942, occasional bushes on lava beds below 100 feet, Villamil, Albemarle Isl., Aug. 29, 1905-1906 (type, Gray: isotypes, Chi., N.Y.); *Stewart* 1,943, low bushes in disintegrated pumice near the shore, Cowley Bay, Albemarle Isl., Aug. 10, 1905-1906 (Chi., Gray, N.Y.); *Stewart* 1,944, occasional bushes 4-5 feet tall on basaltic lava, alt. 850 feet, James Bay, James Isl., Dec. 29, 1905-1906 (Chi., Gray, N.Y.).

The type collection from Villamil (*Stewart* 1,942) is almost entirely staminate, only a stray fruit occurring here and there, and most of the leaves are small, 2 to 3 cm. long and under 1 cm. wide. One sheet of material from Cowley Bay (*Stewart* 1,943, N.Y.) has leaves only slightly larger, but is rich in fruits. The rest of the cited material has considerably larger leaves and might indeed represent a good forma.⁸

DODONAEA ERIOCARPA v *molokaiensis* Degener & Sherff var. nov.—Ramuli ultimi moderate vel conspicue patenti-hispiduli. Folia eis var. *Hillebrandii* subsimilia sed sicca pallidiora, lamina anguste moderate lanceolata apice acuminata principalia 4-6 cm. longa et 0.7-1.7 cm. lata, gracilius longiusque petiolata (petiolo \pm 1.5 cm. longo), faciebus marginibusque glabrata vel debiliter brevissimeque hispidula. Florum feminorum sepala lineari-oblonga vel subovata, subsparsum pubescentes; ovariis dense glandulosi et moderate adpresso-hispidulis setulis simplicibus vel interdum stellatis; stylo debili, interdum \pm 7 mm. longo.

⁸ I have broadened the varietal description to include all the specimens, however, and have been deterred from making further segregations by the fact that Stewart apparently found both forms at Cowley Bay and construed them as identical, distributing them under the same number (1,943). They should be examined in the field. We may note here that the Galapagean flora offers numerous instances of modification into formae. Thus, for instance, Robinson and Greenman (Amer. Jour. Soc. 50: 138. 1895) listed eight local formae of *Euphorbia viminea* Hook. f.

Fructus maturi plerumque minores, saepius sub 12 mm. alti et (in herbario planati) 15 mm. lati alis (plerumque 2 interdum 3 raro 4) inclusis, moderate vel dense pubescentes.

Ultimate branchlets moderately or conspicuously spreading-hispidulous. Leaves somewhat similar to those of var. *Hillebrandii* but paler in dry state, glabrate or weakly and very shortly hispidulous on surfaces and margins, more delicately and elongately petiolate (petiole \pm 1.5 cm. long), principal ones with a blade 4-6 cm. long and 0.7-1.7 cm. wide, narrowly or moderately lanceolate, the tip acuminate. Pistillate flowers: sepals linear-oblong or subovate, subsparingly pubescent; ovaries densely glandular and moderately appressed-hispidulous, their setulae simple or at times stellate; style slender, at times \pm 7 mm. long. Mature fruits commonly smaller, more often under 12 mm. tall and (when flattened in the herbarium) 15 mm. broad including the wings (these commonly 2 sometimes 3 rarely 4), moderately or densely pubescent.

Specimens examined (all from SOUTHEASTERN MOLOKAI): *Otto Degener* 11,165, in one of dry valleys between Kamalo and Kaunakakai, July 29, 1928 (type, Chi.: isotypes, Arn., Berl., Calif., Del., Flor., Gray, Kew, Par.); *Degener* 11,169, small tree, in second eastern gulch, arid region, Wawaia, June 27, 1928 (Arn., Berl., Chi., Kew, N.Y., Par., U.S., etc.).

DODONAEA ERIOCARPA ξ *Hillebrandii* var. nov.; J. F. Rock, Indig. Trees Haw. Isls. pl. 109. 1913. —Folia moderate vel anguste (interdum subrhomboidae) oblanceolato-linear, plerumque 3.5-5 (raro usque ad 6.5) cm. longa et 8-13 mm. lata, infra medium in petiolum brevem sensim angustata apice subacuminata acutave, tenuiora, demum plerumque glabrata. Ramuli moderate vel aegre pubescentes. Fructus demum aegrius pubescentes hispidulive; juvenes ovariave plerumque densissime glandulosa sed irregulariter et tantum substellate pubescentia.

Leaves moderately or narrowly (sometimes subrhomboidally) oblanceolate-linear, commonly 3.5-5 rarely up to 6.5) cm. long and 8-13 mm. wide, gradually narrowed below middle into a short petiole, at apex subacuminate or acute, thinner, finally more often glabrate. Branchlets moderately or weakly pubescent. Fruits at last more weakly pubescent or hispidulous; young ones or ovaries usually very densely glandular but irregularly and only substellately pubescent.

Specimens examined: ISL. MAUI (probably East Maui only)—*H. M. Curran* 44, Isl. Maui, Apr., 1911 (U.S.); *Otto Degener* 2,320, in scrub vegetation, often fog-swept, hill mauka of Olinda, June 15, 1927 (Chi., N.Y.); *Degener* 2,321, within Haleakala, Aug. 16, 1927 (Berl., Chi., Mo., N.Y., Par., Phila., U.S.); *Degener* 2,322, aa lava plain, within Haleakala near Koolau Gap, Aug. 15, 1927 (Berl., Brit., Calif., Chi., Del., Gray, Kew, Minn., Mo., N.Y., Par., U.S.); *Degener* 2,326, dry aa lava, near Kaupo Gap, within Haleakala, Aug. 20, 1927 (Berl., Calif., Chi. (where numerous small

fruiting inflorescences are aggregated into a panicle 2.1 dm. wide and 1.7 dm. tall); Gray, Par.); *Degener* 11,168, within Haleakala, Jan. 20, 1928 (Chi., Kew, Par.); *Degener* 12,487, in scrub, outer slope of Haleakala below observatory, July 25, 1939 (Arn., Berl., Brit., Calif., Chi., Corn., Del., Flor., Gray, Kew, Minn. Mo., Mun., N.Y., Par., Phila., U.S., etc.); *Degener, Ordoñez, & Salucop* 12,489, aa lava plain at 6,000 feet alt., Kaupo Gap, Haleakala, Aug. 11, 1939 (Arn., Brit., Calif., Chi., Del., Gray, Minn., Mo., N.Y., Par., U.S.); *Degener & Henry Wiebke* 2,323, on dry aa lava, near Kaupo Gap in Haleakala Crater, Aug. 20, 1927 (N.Y., U.S.; also at Calif., but with collectors' no. changed to 2,326); *Abbé Urbain Faurie* 1,127, alt. 1,500 meters, Haleakala, Aug., 1909 (Arn.); *Faurie* 1,128, Haleakala, Aug., 1909 (Arn.); *Charles N. Forbes* 288-M, on Crater of Haleakala, Aug., 1910 (Arn., Bish., Calif., Chi., Mo., N.Y., Par., U.S.); *Forbes* 1,107-M, Kaupo Gap, Crater of Haleakala, Aug. 10, 1919 (Bish.); *Forbes* 1,108-M, Kaupo Gap, Aug. 10, 1919 (Bish., Par.); *Forbes* 2,172-M, Puu Ouli, south slope of Haleakala, Apr. 8, 1920 (Bish., Chi.); *F. Raymond Fosberg* 9,970, bush 1.5 meters tall, dry, brushy slope, alt. 2,275 meters, slopes of Haleakala between top of Halemanu Trail and end of road at Puu Nianai (Nianiau), Aug. 23, 1933 (Chi., etc.); *Fosberg* 9,996, bush 1.5 meters tall, on lava bed, alt. 2,240 meters, Haleakala Crater floor, Halemanu Trail between Bottomless Pit and foot of pali, Aug. 23, 1933 (Chi., etc.); *William Hillebrand*, alt. 6,000–9,000 feet, Mt. Haleakala, *commun. anno* 1865 (type, Gray); *Hillebrand & Rev. John M. Lydgate*, Haleakala (Bish.); *Horace Mann & William T. Brigham* 239, Haleakala (Gray, Mo., U.S.); *Joseph F. Rock*, Haleakala, 1912 (Bish., Chi., etc.); *Harold St. John* 10,342, bush 5 feet tall, rocky slope, alt. 8,500 feet, Haleakala, Feb. 15, 1930 (Bish.).

ISL. HAWAII—*C. N. Forbes* 220-H, near summit of Hualalai, June 20, 1911 (Bish.); *Forbes* 754-H, Lava Flow of 1855 below Holealohu (Hualoa), June 7, 1915 (Mo.).

The type had been received at Harvard University in July, 1865, with the data as to habitat of collection but evidently without Hillebrand's determination. Later (Fl. Haw. Isls. 88. 1888), Hillebrand construed this material as a variant of *Dodonaea eriocarpa*: "Maui! Haleakala, 6,000–8,000 ft. (leaves mostly glabrate)." As noted in my description, however, the Haleakala material stands out from the var. *typica* in several respects. It apparently is best interpreted as merely another one of the extremely local varieties for which the Hawaiian Islands are so famous. Specimens of var. *Hillebrandii* sometimes are only with difficulty distinguishable from those of *D. viscosa* var. *spatulata* (Smith) Benth.

DODONAEA ERIOCARPA o *Waitziana* (Blume) comb. nov.; *Dodonaea Waitziana* Blume, Rumphia 3: 189. 1847; *Dodonaea viscosa* var. *Waitziana*

(Bl.) O. Ktze. Rev. Gen. 1: 143. 1891.

Blume's type had been collected "in sylvis montium ignivomorum Javae orientalis." From Blume's ample description we extract: "ramulis junioribus . . . racemis subhirs . . . qua adeo ad *D. eriocarpam* Sm. ex insulis Sandwich proxime accedit . . . petiolis . . . dorso . . . puberulis." Blume described the leaves as 3–5.5 inches long and 0.5–1.33 inches wide, lanceolate, very acuminate, very rarely obtuse or retuse, etc. The three specimens now before me and cited below have leaves essentially as described by Blume, but vary from oblong- to elliptic-lanceolate. They suggest those of *D. viscosa* var. *vulgaris* f. *Schiedeana* (Schlecht.) Radlk. Otto Kuntze (*loc. cit.*) did indeed reduce Blume's species to varietal rank under *D. viscosa*. However, the stellate nature showing more or less definitely in the pubescence reveals a closer affinity with *D. eriocarpa* Sm., as implied by Blume, and accordingly I have renamed the plants *D. eriocarpa* var. *Waitziana*. A very few Hawaiian specimens of *D. eriocarpa* var. *Hosakana* (e.g., *Degener* 11,160) approach this variety in leaf characters but differ sharply in their smaller, more compact, often subglobose clusters of usually smaller fruits.

Specimens examined (all from ISL. JAVA): *Otto Kuntze* 5,872, alt. \pm 5,000 feet, Wilis Mts., 1875 (N.Y.); *W. A. & C. B. Setchell*, Mt. Papandajan, Jan. 30, 1927 (Calif.); *C. G. G. J. van Steenis* 7,199, Pasoeroean (Arn.).

DODONAEA ERIOCARPA π *minor* var. nov.—Unicus ramulus sub 2 dm. longus plus minusve glabratus visus. Folia tenuiora, oblanceolata, apice attenuata acuminatave, infra medium in petiolum brevem (\pm 2 mm.) caespitoso- vel stellato-setulosum elongate attenuata, alibi subglabrata. Unica inflorescentia circ. 3.5 cm. longa et 2.5 cm. lata visa; vix ad anthesin, paniculata, masculina, axe ramulisque subtilibus setulosisque setulis simplicibus vel basi ramosis. Sepala ovata, apice breviter attenuata, extus sparsissime intus marginibusque conspicue hispidula, circ. 2 mm. longa, antheris paulo brevioribus.

A lone branchlet seen, this more or less glabrate and under 2 dm. long. Leaves thinner, oblanceolate, apically attenuate or acuminate, elongately narrowed below middle into a short (\pm 2 mm.), caespitose- or stellate-setulose petiole, elsewhere subglabrate. The solitary inflorescence about 3.5 cm. long and 2.5 cm. broad; this scarcely in flower, paniculate, staminate, its axis and branchlets delicate and setulose, the setulae simple or basally branched. Sepals ovate, at apex shortly attenuate, on outer surface very sparsely but on inner surface and margins conspicuously hispidulous, about 2 mm. long, slightly surpassing the anthers.

Specimens examined: *C. G. G. J. van Steenis* 10,881, alt. 2,100–2,200 meters, west side of Jang Plateau above Taman Hidoep, Besoeki, easternmost Java, July 14, 1938 (type, Arn.).

DODONAEA VISCOSA *minor* var. nov.—Folia valde

coriacea, oblanceolato-oblonga, infra in petiolum 0.5–3 cm. longum angustata, apicem versus obscure repando-marginata apice obtuso vel rotundato abrupte apiculata, lamina usque ad 8 cm. longa et ad 3 cm. lata. Sepala pro floribus femineis plerumque anguste lanceolata plus minusve 1-nervata, 2–3 mm. longa; pro floribus masculinis ovata et sub-3-nervata. Stylus 2–3-partitus, circ. 3–3.5 mm. longus. Capsulae maturae non visae; submaturae alis inclusis ± 1.5 cm. latae.

Leaves very coriaceous, oblanceolate-oblong, narrowed below into a petiole 0.5–3 cm. long, obscurely repand-margined toward and abruptly mucronulate at the obtuse or rounded tip, blade up to 8 cm. long and to 3 cm. wide. Sepals of pistillate flowers commonly narrow-lanceolate, more or less 1-nerved, 2–3 mm. long; those of staminate flowers ovate and subtrinnervate. Style 2–3-parted, about 3–3.5 mm. long. Mature capsules not seen; submature ones ± 1.5 cm. wide including wings.

Specimens examined: J. P. Chapin 903, tree 10 feet tall, trunk diameter 4 inches, flowers small and green, at upper edge of woods, alt. 600 feet, slope of mountain, Isl. Rapa, Austral Isls., Dec. 8, 1934 (type, N.Y.: isotype, Chi.; pistillate, subfruiting material); Chapin 909, tree 18 feet tall, trunk diameter 6 inches, flowers greenish, stamens yellow, often red on outer side, alt. 550 feet, bushy slope of mountain, Isl. Rapa, same date (N.Y.).

Of much smaller geographic range (hence the name *minor*) than the other known varieties of *D. viscosa*.⁹

Recently Forest Brown (Bish. Mus. Bull. 130: 164. 1935) described *D. viscosa* var. *Stokesiana* from the Austral Islands, among them Rapa. For that variety the leaf-petioles were described as short, ± 2 mm. in length. Var. *minor* has much longer petioles. Of Brown's cited material of var. *Stokesiana*, a lone, numerous staminate-flowered specimen (Stokes 191) from Rurutu is before me. Its sepals average more nearly ovate and more distinctly 3-nerved than in the observed pistillate material of var. *rapensis*. Pistillate specimens of the two varieties should be compared when available.

In the type of var. *minor*, various straight or straightish, white hairs occur irregularly in the inflorescence, these sometimes clustered or stellately arranged. They appear now natural now almost as if derived by crystal-formation or by deposition from some foreign source.

DODONAEA STENOPTERA var. **Fauriei** (Lévl.) comb. nov.; \times *Dodonaea Fauriei* Lévl. in Fedde Repert. Spec. Nov. 10: 155. 1911.—*Dodonaea Fauriei* was described as a new hybrid, "*D. viscosa*

⁹ Mention may be made here of *Dodonaea Candolleana* var. *minor* Blume (Rumphia 3: 191. 1847), based on material from Java, etc. Blume's description was scanty, and under present war conditions there is little hope of consulting original specimens with a view to learning just what his var. *minor* was. I suspect, however, that it will be found to belong somewhere in *D. viscosa* or *D. eriocarpa*.

L. \times *D. stenoptera* Hillebr.," and was based on *Faurie* 299, Waianae [Waianae], westernmost Oahu, May, 1910. Specimens of the type collection (Bish., Par., etc.) are before me. They have many fruits, these immature and with mostly two but sometimes three very narrow wings, suggesting indeed *D. stenoptera* Hillebr., a species known only from southeastern Molokai. Léveillé clearly assumed that the narrowness of the fruiting wings was traceable to a *D. stenoptera* parentage while the fewness of these wings (mostly two, not four as in *D. stenoptera*) was traceable to a *D. viscosa* parentage (many Hawaiian specimens, most of them erroneously known to him as *D. viscosa*, having two-winged fruits). But since true *D. stenoptera* is known only from Molokai and, moreover, only from the far side of the island, there could have been little chance for it to produce a hybrid on Oahu. Rock (in Fedde Repert. Spec. Nov. 13: 352. 1914) called *D. Fauriei* Lévl. a "deformed specimen" of *D. viscosa* L. However, Fosberg collected an excellent suite of specimens in essentially the same locality on Oahu 23 years after *Faurie* had collected there, and the Fosberg plants show the same fruit characters as did the *Faurie* plants. There seems no reason, therefore, to regard the fruits as indicating any deformity. Rather do they appear to indicate a varietal rank for the Oahu material, under *D. stenoptera* Hillebr. In the *Faurie* plants, the material is pistillate, with here and there rudiments of stamens present. In the Fosberg plants, some of the pistillate flowers have a full complement of stamens, these in certain cases appearing as if functional.

Specimens examined: Abbé Urbain *Faurie* 299, Waianae, Isl. Oahu, 1910 (type coll., Bish., Par., etc.); F. R. Fosberg 9,494, tree 4 meters tall, both staminate and pistillate flowers on same tree, alt. 650 meters, in dry forest, Halona Valley, Waianae Mts., Lualualei, Isl. Oahu, May 12, 1933 (Chi., etc.).

DODONAEA STENOPTERA Hillebr. Haw. Isls. 88. 1888.—In the foregoing study of *D. stenoptera* var. *Fauriei*, an examination was made of all available specimens of the species proper, which for convenience of reference may be designated var. **typica** var. nov. It may be noted that Hillebrand studied *D. stenoptera* in considerable detail, describing even the seed-embryo. He termed it "a very distinct species." Rock (Indig. Trees Haw. Isls. 281. 1913) accepted *D. stenoptera* as a species and stated that it grew above Kamalo on Molokai. The following specimens have been studied by me:

Specimens examined (all from MOLOKAI): *Faurie* 274, alt. 1,000 meters, Kamalo, June, 1910 (Arn., Bish., Del., Par.); Charles N. Forbes 127-Mo, on ridges below Puu Kolekole, July, 1912 (Bish., Mo., N.Y.); William Hillebrand, alt. 1,500–2,000 feet, Kamalo (Gray, Kew); Albert S. Hitchcock 15,107 alt. about 4,000 feet, north of Kamalo,

Oct. 10, 1916 (Bish., U.S.); *Rock*, Kamalo, Feb., 1920 (Arn.).

In a forthcoming and considerably longer article upon *Dodonaea*, it is found necessary to use certain new names and new combinations. These are published at this time for the purpose of properly validating them:

DODONAEA VISCOSA var. **linearis** (Harv. & Sond.) comb. nov. et f. **linearis** f. nov.; *Dodonaea linearifolia* Linden Pl. Cub. num. 2,070 (annis 1841-1846) et apud Turcz. Bull. Mosc. 31¹: 407. 1858; *Dodonaea linearis* E. Mey. in Hb. Drège, Flora 26^{II}, Beigabe p. 179. 1843; *Dodonaea Thunbergiana* var. **linearis** Harv. & Sond. Fl. Cap. 1: 242, sub num. 2. 1859 & 1860.

DODONAEA VISCOSA var. **linearis** f. **angustifolia** (Benth.) comb. nov.; *Dodonaea angustifolia* L.f. Suppl. Pl. Syst. Veg. 218. 1781; *Dodonaea angustifolia* Swartz, Observ. Bot. 150. 1791; *Dodonaea viscosa* var. **angustifolia** Benth. Fl. Austral. 1: 476. 1863.

DODONAEA VISCOSA var. **linearis** f. **arizonica** (A. Nels.) comb. nov.; *Dodonaea arizonica* A. Nels. Amer. Jour. Bot. 21: 576. 1934.

DODONAEA VISCOSA var. **arborescens** (Cunn.) comb. nov. et f. **arborescens** f. nov.; *Dodonaea arborescens* Cunningham ex W. J. Hook. Jour. Bot. 1: 251. 1834 (as a syn. for *D. Aspleniifolia* var. β W. J. Hook. loc. cit.); *Dodonaea Aspleniifolia* var. **arborescens** (Cunn.) J. D. Hook. ex W. J. Hook. op. cit. 2: 415. 1840.

DODONAEA VISCOSA var. **arborescens** f. **spatulata** (Sm.) comb. nov.; *Dodonaea spatulata* J. E. Smith in Rees Encyclop. 12: no. 2. 1809; *Dodonaea viscosa* var. **spatulata** (Sm.) Benth. Fl. Austral. 1: 476. 1863; *Dodonaea viscosa* f. **arborescens** Hert. Rev. S. Amer. Bot. 3: 168. 1936. *Nomen*; *Dodonaea arborescens* Hert. op. cit. 5: 35. 1937.

DODONAEA VISCOSA var. **arborescens** f. **Ehrenbergii** (Schlecht.) comb. nov.; *Dodonaea Ehrenbergii* Schlecht. Linnaea 17: 639 (sphalm 739). 1843; *ibid.* 18: 36 (sphalm 52). 1844; *Dodonaea viscosa* var. **obovata** Hitchc. Report. Missouri Bot. Gard. 4: 73. 1893; *Dodonaea microcarya* Small, Torreya 25: 38. 1925.

CHICAGO TEACHERS COLLEGE,
CHICAGO, ILLINOIS

THE PRODUCTION AND CHARACTERIZATION OF ULTRAVIOLET-INDUCED MUTATIONS IN *ASPERGILLUS TERREUS*. III. BIOCHEMICAL CHARACTERISTICS OF THE MUTATIONS ¹

Lewis B. Lockwood, Kenneth B. Raper, Andrew J. Moyer, and Robert D. Coghill

PREVIOUS ANALYSES and descriptions of the effects of irradiation of conidia with ultraviolet light have dealt almost entirely with obvious gross morphological or cultural changes which are readily observable. The more difficult physiological and biochemical characterizations of light-induced variants have only recently been undertaken. Thus, Tatum and Beadle (1942) have obtained strains of *Neurospora* which had lost the ability to meet their own requirements of thiamin, thiazol, nicotinic acid, pantothenic acid, pyridoxin, methionine, lysine, arginine, leucine, and tryptophane. Raper, Coghill, and Hollaender (1945) have described two biochemical variants of *Aspergillus terreus*. One of these required an external source of thiamin for growth but was indistinguishable from the parent strain when an adequate supply of thiamin was present. The other strain had lost the ability to metabolize nitrates but made normal growth when ammonium ion was present.

While two of us (Moyer and Coghill, 1945) were engaged in a study of the production of itaconic acid from glucose, it became evident that a considerable portion of the glucose metabolized was converted into products other than itaconic acid. With the objective of obtaining mutant strains of

the fungus in which some of the enzyme systems were destroyed, irradiation experiments were conducted in cooperation with Dr. Alexander Hollaender of the National Institute of Health, Bethesda, Maryland. Data obtained in these experiments are presented by Hollaender, Raper, and Coghill (1945). It was hoped that the elimination of competing enzyme systems would result in the conversion of a larger percentage of the sugar to itaconic acid. Since no correlation of morphological characters with the physiological properties of *Aspergillus terreus* was known, it was necessary that both obvious morphological variants and strains which morphologically resembled the parent culture be selected for biochemical testing. Of the large number of cultures derived from irradiated conidia, a random selection of 217 strains was made for such examination.

CULTURE CONDITIONS AND ANALYTICAL METHODS.—A culture medium developed previously by one of us (A. J. M.) was used for the biochemical studies of all the strains of *A. terreus*. It had the following composition:

Glucose monohydrate (commercial).....	250	g.
NH ₄ NO ₃	2.47	g.
MgSO ₄ ·7H ₂ O	0.25	g.
KCl	0.1	g.
ZnSO ₄ ·7H ₂ O	0.0024	g.
HNO ₃ (sp. g. 1.42)	1.60	ml.
Concentrated corn steep liquor.....	4.0	ml.
Distilled water to	1.0	liter

¹ Received for publication December 18, 1944.

The authors wish to express their grateful appreciation for analytical services to Morris Friedkin, Max D. Reeves, and Mrs. Lucille B. Czaplá of this laboratory.

The medium was distributed in 60-ml. portions in 200-ml. Pyrex Erlenmeyer flasks, and sterilized in an autoclave for 20 minutes at 120° to 121°C. (15 to 16 lbs. steam pressure). The pH of the cooled medium was approximately 1.8. The inoculum consisted of spores scraped from malt or Czapek agar slants. All cultures were incubated 12 days at 30°C.

Glucose concentrations were determined by the methods of Shaffer and Hartmann (1921). Total acidities were measured by titrating with standard alkali, phenolphthalein being used as the indicator. Itaconic acid was determined by the bromination method developed in this laboratory by Morris Friedkin and soon to be the subject of another communication (Friedkin, 1945). Total solids were obtained from the Schönrock sucrose table for refractive indices observed with an Abbe refractometer (Assoc. Official Agric. Chem., 1940). The values for neutral nonreducing compounds were obtained by subtracting from the value of total solids the sum of the values obtained for total acidity (calculated as itaconic acid) and residual glucose. Mycelial pads were dried in an oven at 105°C. for 24 hours before being weighed.

RESULTS.—Consideration of the cultural and biochemical data obtained from these studies has led to division of the 217 strains into several groups. One hundred and one strains showed no change in any of the biochemical characteristics studied, although 42 of these strains were obviously altered morphologically. One hundred and sixteen strains showed some type of biochemical alteration ranging from failure to grow on the test medium to increased growth and greater acid production than the parent strain. Although 99 of these mutants showed various degrees of modification in morphology, no consistent relationship between bio-

chemical and morphological mutation was observed. However, strains which produced colonies characterized by limited yellow floccose growth with few spores generally formed little acid (Raper's type V) as discussed by Raper, Coghill, and Hollaender (1915). The mutants are conveniently distributed among nine groups. Groups one and two include all those organisms which were apparently unaltered biochemically, and the remaining seven groups segregate the 116 biochemically variant strains according to different types of cultural or biochemical response. Since the differentiation of these groups is not always based on the same characteristics, some strains appear in more than one category.

Group 1.—This group of 59 strains was indistinguishable from the parent nonirradiated strain in any of the characteristics which were studied.

Group 2.—This class was made up of 42 morphologically or culturally variant strains which were similar to the nonirradiated parent strain in the efficiency of conversion of glucose into both total acid and itaconic acid. Some of these strains were characterized by loss of spore color, type of growth or colonial form on Czapek agar, different from the parent strain.

Group 3.—This group comprised 13 strains which, with two exceptions (Raper, Coghill, and Hollaender, 1945), were morphologically and culturally indistinguishable from the parent nonirradiated strain, but which converted glucose to acid with greater efficiency. The ratios between total acid and itaconic acid were approximately the same as in the parent strain.

Group 4.—This category consisted of only four strains which were morphologically and culturally indistinguishable from the parent nonirradiated

TABLE 1. Biochemical characteristics illustrating the reactions of representative strains of nine groups of mutant strains of *Aspergillus terreus* obtained by irradiation. Data are averages of triplicate cultures except for non-irradiated parent strain where 54 cultures are averaged.

Group	Number of strains	Number of representative strain	Glucose consumed per culture	Total acid per culture	Itaconic acid per culture	Itaconic acid per culture	Purity	Itaconic acid yield ^a based on glucose		Neutral non-reducing compounds per culture	Mat weights per culture
								Con- sumed	Sup- plied		
			Grams	Ml. of N/10	Ml. of N/10	Grams	Per cent	Per cent	Per cent	Grams	Grams
		Nonirradiated parent strain	11.4	445	411	2.67	92.2	32.4	25.4	1.6	1.927
1	59	30-6-8	12.2	451	405	2.63	89.7	29.8	23.7	1.3	2.010
2	42	32E-SS-1-1	12.4	417	397	2.57	95.2	28.9	27.2	1.0	1.964
3	13	32D-S-4-2	11.4	497	468	3.06	94.2	37.2	29.4	2.1	1.703
4	4	32A-SS-1-19	10.9	51	2.2	2.147
5	88	C	2.5	22	0.411
6	15	30-5-1	6.5	226	290	1.47	128.0	31.3	13.4	0.8	0.875
7	17	29-6-4	10.5	323	324	2.10	100.3	27.4	20.0	2.1	2.216
8	15	32A-SS-3-4	13.7	27	4.0	2.595
9	11	32F-SS-4-1	0	0

^a Theoretical yield, assuming that one mol of glucose yields one mol of itaconic acid.

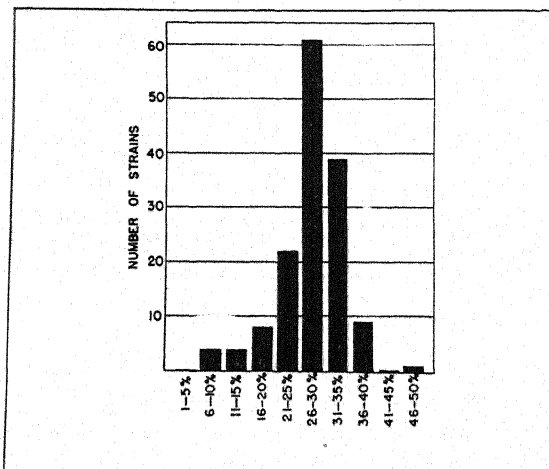
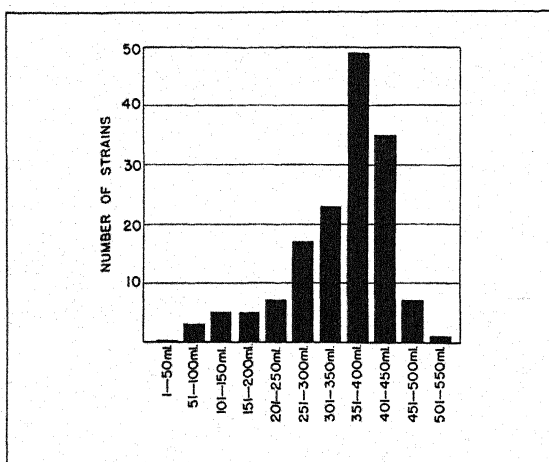
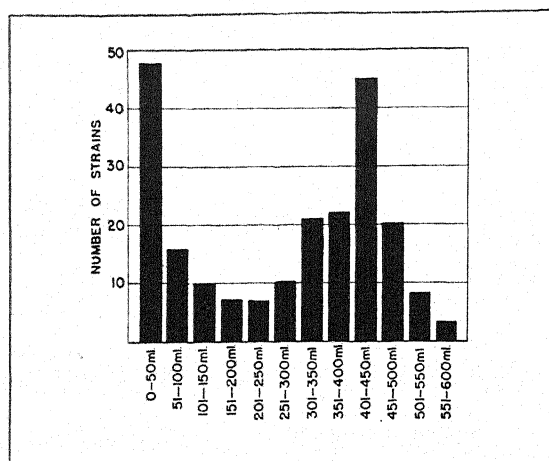


Fig. 1 (upper). Distribution of *Aspergillus terreus* mutants—Total Acid.—Fig. 2 (middle). Distribution of *Aspergillus terreus* mutants which produced at least 50 ml. total acid per culture—Itaconic Acid.—Fig. 3 (lower). Per cent yield itaconic acid (theoretical), based on glucose consumed by *Aspergillus terreus* mutants.

strain, but which produced little or no acid from glucose.

Group 5.—This group comprised 88 obvious morphologically or culturally mutant strains which produced little or no acid from glucose. A few of these grew poorly on the test medium, and in all cultures the efficiency of conversion of glucose to acid was very low.

Group 6.—This group consisted of 15 strains in which the apparent purity of the acid produced was considerably in excess of 100 per cent. The fact that the values for unsaturated linkages, as determined by bromination, are in excess of the theoretically equivalent values for itaconic acid, as measured by alkali titration, is indicative of the presence of a considerable quantity of nonacidic unsaturated material. These cultures were always highly pigmented.

Group 7.—This group comprised 17 strains which, judging from the ratio between total acidity and itaconic acid as determined by bromination analysis, produced substantially only itaconic acid. None

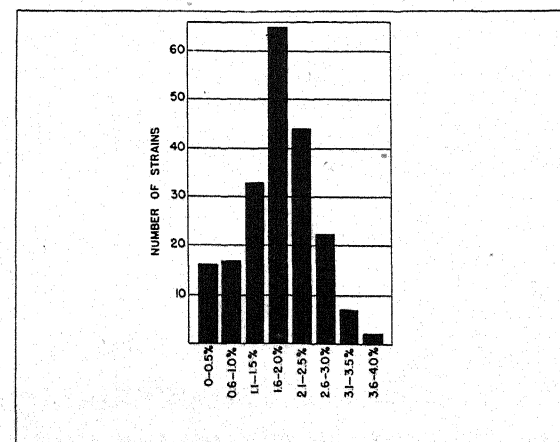
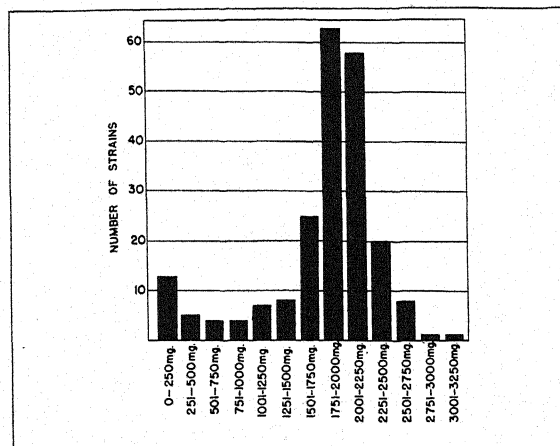


Fig. 4 (upper). Mat weights—*Aspergillus terreus* mutants.—Fig. 5 (lower). Neutral nonreducing materials in the culture liquor of *Aspergillus terreus* mutants.

of these cultures produced much pigment, whereas in the preceding group heavy pigment formation was associated with high bromination values.

Group 8.—This group consisted of 15 strains which produced considerably more neutral non-reducing compounds from glucose than did the non-irradiated parent strain. Three of the strains of group 6 also were classified in this group. No relationship between the production of neutral non-reducing compounds and acids was observed.

Group 9.—This group consisted of 11 cultures which failed to grow on the test medium. Since these strains all grew readily on Czapek agar slants, and the test medium contained a crude vegetable extract (corn steeping liquor), lack of growth was not due to a deficiency of growth factors. Few of these strains sporulated readily, and inoculations were usually made by mycelial transfer. It is possible that the resistance of these strains to acidity was not great enough to permit growth at the low initial pH of the test medium.

Data obtained from the triplicate tests of individual strains selected as illustrative of the above groups are presented in table 1. For comparison, average values from 54 test cultures of the non-irradiated parent strain are presented.

Block graphs show the distribution of irradiated strains with respect to total acid production (fig. 1); itaconic acid production (fig. 2); efficiency of conversion of glucose to itaconic acid (fig. 3); mycelial weights (fig. 4); and the production of neutral non-reducing materials (fig. 5). It is of interest to note that the nonirradiated parent strain would appear in the longest block of each graph, except for total acid production (fig. 1), thus indicating that a large part of the strains isolated are not greatly altered, according to the biochemical criteria used here. When considered in terms of total acidity, approximately equal numbers of cultures showed no alteration, or the nearly complete failure to accumulate acid. The large number of strains of this latter type indicates the ease with which the itaconic acid-producing enzyme system is destroyed. Concurrent with the survey of isolates resulting from irradiated spores of strain NRRL 265, a similar survey was made of strains of *A. terreus* newly isolated from nature. Among such isolates, yields of itaconic acid were low in many strains, approximately equal to nonirradiated NRRL 265 in others, and in a very limited number of isolates were substantially higher than in any of the mutants of NRRL 265 produced by ultraviolet light. These newly isolated, high-yielding strains afford especially favorable material for further radiation studies.

From the point of view of developing a practical fermentation process, the few strains which produced more acid than did the parent nonirradiated strain are especially important. They represent a

preferential channelling of metabolism through the enzyme system responsible for the production of itaconic acid.

SUMMARY

Nine different types of biochemical and cultural response have been observed from 217 strains of *Aspergillus terreus* derived from irradiated conidia.

Among the 76 strains which were morphologically unchanged were 59 that appeared to be unaltered biochemically, 13 that produced more itaconic acid than the parent strain, and 4 that produced no itaconic acid.

Among the 141 strains which were obviously altered morphologically were 42 strains not apparently altered biochemically, 88 which produced little acid, and 11 which did not grow on the test medium. None of these 141 strains produced more itaconic acid than did the parent strain.

Fifteen strains produced considerable nonacidic unsaturated material.

Seventeen strains appeared to produce no acid other than itaconic.

The distribution of strains, plotted in terms of total acid produced, itaconic acid produced, efficiency of conversion of glucose to itaconic acid, mycelial weights, and neutral nonreducing materials produced, is presented.

FERMENTATION DIVISION,

NORTHERN REGIONAL RESEARCH LABORATORY,

BUREAU OF AGRICULTURE AND INDUSTRIAL

CHEMISTRY,

AGRICULTURAL RESEARCH ADMINISTRATION,

U. S. DEPARTMENT OF AGRICULTURE,

PEORIA, ILLINOIS

LITERATURE CITED

- ASSOC. OFFICIAL AGRIC. CHEM. 1940. Official and tentative methods of analysis. 5th ed. p. 425. Washington.
- FRIEDKIN, MORRIS. 1945. Determination of itaconic acid in fermentation liquors. (In preparation.)
- HOLLAENDER, A., K. B. RAPER, AND R. D. COGHILL. 1945. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. I. Production of the mutations. Amer. Jour. Bot. 32: 160-165.
- MOYER, A. J., AND R. D. COGHILL. 1945. The laboratory scale production of itaconic acid by *Aspergillus terreus*. (In preparation.)
- RAPER, K. B., R. D. COGHILL, AND A. HOLLAENDER. 1945. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. II. Cultural and morphological characteristics of the mutations. Amer. Jour. Bot. 32: 165-176.
- SHAFFER, P. A., AND A. F. HARTMANN. 1921. The iodometric determination of copper and its use in sugar analysis. II. Methods for the determination of reducing sugars in blood, urine, milk, and other solutions. Jour. Biol. Chem. 45: 365-390.
- TATUM, E. L., AND G. W. BEADLE. 1942. The relation of genetics to growth factors and hormones. Growth (Fourth Growth Symposium) 6 (supplement): 27-35.

QUANTITATIVE IRRADIATION EXPERIMENTS WITH *NEUROSPORA CRASSA*.

I. EXPERIMENTS WITH X-RAYS¹

Eva R. Sansome, M. Demerec, and Alexander Hollaender

IN QUANTITATIVE experiments on the effect of ultraviolet radiation in inducing mutations, one of the greatest problems is the high degree of absorption of the rays by the cytoplasm. This has been shown very clearly by the work of Stadler and Uber on pollen grains of *Zea* (Stadler and Uber, 1942). In fungal spores, where the treated objects are small and relatively uniform in size, this difficulty is at a minimum. Moreover, observations on large numbers of individuals can often be obtained in a relatively short time. A certain amount of work has been done on the effect of ultraviolet in inducing variants in *Trichophyton mentagrophytes* (Emmons and Hollaender, 1939). Since this fungus has no known sexual stage, however, the variants could not be subjected to genetical analysis. It was therefore decided to repeat the work with *Neurospora crassa*, an Ascomycete. Its life history had been studied in detail by Shear and Dodge (1920), who found that it is heterothallic and that its sexual reproduction is readily controlled experimentally. Lindegren (1942), Dodge (1930), and others had investigated its inheritance and found that Mendelian segregation occurs in the ascus. In addition, this fungus had already been used in radiation experiments (Lindegren and Lindegren, 1941). The number of spores that can be isolated in any experiment on *N. crassa* is limited, because of the rapid, spreading growth of this fungus. It was felt, however, that this disadvantage was outweighed by the possibility of analyzing the variants genetically.

Experiments on the effects of X-radiation were undertaken to parallel the ultraviolet experiments (Hollaender, Sansome, Zimmer and Demerec, 1945), since it was thought that a comparison of the effects of these two agents might give some indication as to how they act in inducing mutations. Such comparisons have been tried before, but the absorption difficulty in the case of ultraviolet radiation has always interfered with quantitative work using this agent.

MATERIALS AND METHODS.—The methods employed were essentially those described by the Lindegrens (1941). Microconidia were used for these experiments, because their uninucleate condition, small size, and uniformity render them very suitable for ultraviolet treatment. They were obtained from the fluffy mutant, which does not produce macroconidia but only microconidia. The use of fluffy served also as a check against contamination, since *Neurospora* contaminants were more likely to be of the wild conidial type.

The cultures were passed through a single ascospore or single microconidium stage, not more than

three weeks previous to the treatments. This was done to reduce the chance of storing up spontaneously occurring mutations. Seven- to ten-day-old fluffy cultures were wetted down with sterile water. Two days later, microconidia, or microspores, could be seen as a pink, powdery film on the surface of the liquid. The microconidia were washed off with sterile saline solution and filtered through absorbent cotton under sterile conditions. The absorbent cotton holds back the pieces of mycelium but lets the small microconidia pass through.

The spores were then centrifuged, and a concentrated suspension of spores (about 5×10^6 per cc.) was X-rayed in a small, thin-walled glass tube. The irradiation was carried out at the laboratory in Cold Spring Harbor, or, when high intensity was required, at the Memorial Hospital in New York City.² At Cold Spring Harbor, dosage was measured by a Victoreen dosimeter, by placing the chamber adjacent to the vial with the spores. In order to compensate for absorption by the glass of the vial, a piece of glass of equal thickness was placed over the chamber. In treatments made at the Memorial Hospital, dosage was determined by the time of exposure, because the emission of the X-ray tube was standardized. In these experiments no allowance was made for the thickness of the glass; but, since tubes of equal thickness were used, the high-intensity experiments may be considered in relation to one another, although comparisons between the Cold Spring Harbor and the Memorial Hospital experiments must be made with reservation.

Three or four successive spore dilutions were plated out, each dilution being ten times the preceding one. Four to eight plates were prepared for each dilution, by plating 0.3 cc. of spore suspension. The spores were incubated at approximately 22°C., and when they began to germinate after about 48 hours they were isolated from the most suitable dilution plates. In the later experiments a sample of the spore suspension was counted in a blood-counting chamber, as a guide to selecting the most suitable dilutions. Single germinating spores were isolated into tubes of potato-carrot-dextrose agar and incubated at 25°C. The cultures were examined several times during the growing period; and the final classification into mutant and normal phenotypes was made about two weeks after isolation for all cultures except those that grew very slowly, which were classified later. The capacity of the variants to give the mating-type reaction of the original strain was used as an additional check for contaminants in all the earlier experiments, although after a little practice with the material it

² We wish to thank Mr. L. D. Marinelli, of the Memorial Hospital, and Dr. R. S. Anderson, formerly of that hospital, for conducting the high-intensity irradiation.

¹ Received for publication December 23, 1944.

was usually possible to distinguish between contaminants and mutants with considerable accuracy. Furthermore, the spores were handled in a special transfer room, and the amount of contamination was negligible.

All types different from controls and from the treated material were recorded as mutants. The detection of mutants depends partly, of course, upon the personal factor. However, because the spore suspension before treatment consists of identical haploid spores, and the conditions of growth are kept relatively constant—much more constant than is usually possible for higher organisms—the detection of mutant types is comparatively easy. The more uniform the conditions are kept, the easier is the scoring of the mutant types.

OBSERVATIONS. Types of mutants.—The mutants recorded were generally forms differing from the treated type in growth habit. Color changes were also observed, but they usually accompanied morphological changes. In the fluffy strain used for these experiments, color changes are not so striking as in the conidial type, where the pigment is concentrated in the colored spores. Two types of color change were observed, however. In one experiment an albino appeared, which proved to correspond to Beadle's albino-2 (Beadle, personal communication); and several mutants were obtained which produced a dark-brown or black rim at the surface of the medium. A great diversity of growth forms was observed, from tiny colonial forms to types as vigorous as the normal but having special branching systems. The aberrant types were picked out by macroscopic examination; but usually types with aberrant growth habits showed differences in their branching systems when examined under the dissecting microscope.

In one experiment the mutants were classified as follows: 121 with half-to-normal vigor and almost normal growth habit; 29 with half-to-normal vigor and very distinctive growth habit; 39 with some aerial growth, but less than half the normal, and almost normal growth habit; 6 with less than half normal growth and very distinctive growth habit; and 33 with only surface growth. The dosages used in this experiment were 15,750 r and 31,500 r at 5,400 r per minute, and 31,500 r at 270 r per minute. The distribution of the mutants into the various classes was similar for all treatments.

Of 39 cases in which ascospores were grown from crosses between mutants and wild-type, the mutant type was recovered 36 times. In two of the remaining three cases, the mutants reverted to fluffy. There seems little doubt, therefore, that with few exceptions the mutant types involved true genetic changes.

Reversions.—The Lindgrens observed that about half the mutants they obtained in their X-ray experiments reverted to normal after one or several sub-cultures. We also found in our early experiments that some of the cultures recorded at first as mutants later appeared normal. In some cases, mu-

nants were recovered from the offspring of crosses between reversions and normals; in others, only normals were obtained. Such results may be due to several causes. One possibility is that the culture may have been wrongly classified as a mutant. A second possibility is that the culture may have been heterokaryotic from the beginning, carrying normal and mutated nuclei, and that the normal nuclei gradually overcame the effect of the mutant nuclei. Such a heterokaryon could arise by the fusion of mycelia from two spores that had been picked up together; or it could be caused by a binucleate microconidium, or by the fact that only one chromatid of a chromosome was affected by the irradiation. A third possibility is that two chromatid changes might occur and might be distributed to different nuclei. In such a case, two different types of nuclei would occur, each containing one mutant and the normal allele of the mutant in the other type of nucleus. If the mutants were less vigorous than the normal, the heterokaryotic condition would be favored and a balanced heterokaryon would result. It is conceivable that such a balanced heterokaryon might at first appear mutant and later normal, if the relative proportions of the two kinds of nuclei should change and that change should affect the threshold that determines the phenotype (Beadle and Coonradt, 1944). A fourth possibility is that the culture, at first mutant and homokaryotic, may have become heterokaryotic by mutation subsequent to treatment. The mutation may involve the original mutated locus and thus be a true reversion, or it may involve another locus or loci. Such mutation might include chromosome changes, such as the loss during development of a duplication leading to the expression of the mutant character. The fifth possibility is that a type which appears mutant at first may appear normal after considerable vegetative growth, because of some kind of cytoplasmic adaptation compensating for the effect of the mutant gene. In this case it might be expected that the original mutant character would be recovered on sexual reproduction. A sixth possibility—the effect of X-rays on the cytoplasm and the influence of this changed cytoplasm on the appearance of the mutants—has been considered. The possibility of a cytoplasmic effect is thought to be remote.

The problem, then, was how to treat reversions when classifying the cultures as normals or mutants. Experiments with artificial heterokaryons indicated that, when such heterokaryons were eventually normal in appearance, they became so very rapidly. Therefore it was felt that apparent reversions, due to the mutation of only one chromatid of a chromosome, would appear normal very soon and in most cases would never be distinguishable from true normals. Because of the probability that in most cases such types could not be distinguished, it was thought best to exclude them altogether by recording as mutants only those types which appeared mutant two weeks after isolation. Those cultures that appeared mutant at first but changed to

TABLE 1. Data showing acceleration of germination in irradiated spores. Intensity in experiment 11 was 240 roentgens per minute, and in experiment 30 5,400 roentgens per minute.

Treatment	Experiment number	Number of plates	Germination				
			24 hours	Final	Per cent 24 hours	Average per plate	Per cent
Control	11	3	56	216	25.9	72	100
2,250 r	11	2	106	156	67.9	78	108
9,000 r	11	3	102	157	65.0	52	73
13,500 r	11	3	121	198	61.1	66	92
Control	30	4	158	675	23.4	169	100
22,500 r	30	4	274	568	48.2	142	84
31,500 r	30	4	201	423	47.5	106	63

normal at the end of two weeks were classified as normals. It is realized that in some cases reverse mutations or adaptations might have occurred during the two weeks; but these were probably not frequent enough to affect the results seriously, especially as they concern the effects of one dosage relative to another.

Acceleration of germination by X-radiation.—While the main interest of these investigations has been in the mutation rate, it was desired to obtain some idea of the survival rate of treated spores as compared with controls. Since it is extremely difficult to count all the spores germinating on any particular plate, because the early-germinating spores tend to overgrow the plate, it was thought that we might be able to determine the per cent survival by plating out spores at the same time, keeping them under similar conditions and counting the number of germinated spores at a certain given time. When such observations were made, however, it was found that plates with irradiated spores gave higher counts than controls. Attempts were therefore made to obtain complete counts of the germination of control and treated spores and also records of the time of germination. In making the counts, consideration had to be given to the fact that the counting took a considerable amount of time and the plates counted last tended to be farther advanced than those counted first. Various precautions were taken to overcome this difficulty. The counting was staggered so that one plate of each control and treated series was counted as nearly as possible at the same time. While one plate of a set was being counted, the remainder were kept in the refrigerator. Since it was suspected from previous observations that the control spores would germinate more slowly than the treated spores, the controls in each set were counted last, so that any observable difference would not be due to the time of counting.

The results obtained from the two experiments are given in table 1. In experiment 11, 25.9 per cent of the control spores had germinated by the end of the first day of germination, whereas 67.9, 65, and 61.1 per cent of the spores of the three irradiated groups had germinated. In experiment 30, twenty-four hours after the start of germination, when 23.4 per cent of the control spores had

germinated, 48.2 per cent of the spores given 22,500 r units and 47.5 per cent of the spores given 31,500 r units had germinated. It will be seen that, in both experiments, the treated spores germinated earlier than the controls, although in general the ultimate number of germinated spores was highest in the controls. These data are not extensive, but they are supported by observations in other experiments where detailed counts were not made. Thus it seems reasonably certain that X-radiation at dosages from 2250 r to 31,500 r, and at high and low intensities, somewhat accelerates the germination of microconidia of *Neurospora*, when the spores are irradiated in salt solution. There is no evidence of any increase in the effect with increased dosage. Since the acceleration in germination is a transient effect, it is thought that it may be due to some action on the spore membrane or cytoplasm that facilitates intake of water and so hastens germination. This is supported by the fact that after very high dosages, when many of the spores do not germinate, these spores undergo the swelling that precedes germination.

Germination and survival percentages.—Table 2 gives data on the germination and survival-after-germination percentages of controls and treated spores in four experiments. In experiments 11 and 12, the irradiation was given at the laboratory at Cold Spring Harbor, at low intensity (approximately 240 r per minute). In experiments 25 and 30, the irradiation was given at an intensity of 5,400 r per minute at the Memorial Hospital, New York City.

Two types of data are included in table 2. The germination numbers are considered first, and the germination percentage, obtained by relating the actual numbers obtained to the numbers in the controls and counting the control germination as 100 per cent, are given in column 5. Another factor, the survival of spores after germination, is also taken into account. Column 8 gives the survival percentages among the germinated spores, counting the control survival as 100; and column 9 gives the final survival percentage, taking into consideration both failure of germination and failure to grow after germination.

Because of the difficulty of obtaining these data,

TABLE 2. *Germination and survival rates.*

Dosage (r units)	Experiment number	Plates recorded	Germination		Spores isolated	Per cent spores surviving	Per cent survivals of	
			Number spores	Per cent germination (con- trol = 100)			Germinated (con- trol = 100)	Sown (con- trol = 100)
Intensity 240 r per minute								
Control	11	3	216	...	115	91.2
9,000	11	3	157	72.2	139	91.4	100.2	72.3
13,500	11	3	198	91.7	172	88.4	96.9	88.9
Control	12	4	159	...	60	96.7
4,500	12	3	123	102.5	139	92.1	95.2	97.6
13,500	12	4	130	80.0	134	94.8	98.0	78.4
31,500	12	4	100	62.5	105	81.0	83.8	52.4
Intensity 5,400 r per minute								
Control	30	4	674	...	144	96.5
22,500	30	4	568	84.3	376	73.1	75.8	63.9
31,500	30	4	423	62.8	344	61.0	63.2	39.7
Control	25	3	595	...	116	94.
31,500	25	3	289	48.6	236	52.5	55.9	27.2
63,000	25	3	762 ^a	12.8	228	15.4	16.4	2.1
126,000	25	3	337 ^a	0.57	250	2.4	2.6	0.01

^a Lower dilution than preceding line counted.

and the possibilities of error, they must be regarded with caution. Nevertheless, it seems clear that these microconidia are quite resistant to X-radiation, since more than half of them survive a dose of 31,500 r units. It is interesting to compare the survival values obtained in this way with those of Lindegren and Lindegren (1941). When adjustment is made for the fact that the Lindegrens' results are direct results and ours are based on the assumption of 100 per cent germination in the controls, their survival percentages are 75 per cent for 13,750 r units and 53 per cent for 22,000 r units, which is in fair agreement with our values of 78.4 per cent for 13,500 r and 63.9 per cent for 22,500 r. At the highest dosage given, 126,000 r units, only 0.01 per cent of the spores survived. At 31,500 r units, for which we have both high- and low-intensity data, it seems that the germination percentages, being 48.6 and 62.8 in the high-intensity series and 62.5 in the low-intensity series, are approximately the same, regardless of the intensity. There is some indication that the survival percentage is lower in the high-intensity series, 62.2 per cent and 55.9 per cent, as compared with 83.8 per cent in the low-intensity treatment. The survival after germination is easy to determine and less subject to error than the germination percentage. Therefore it seems likely that this is a real difference. Thus, while there is no evidence that the intensity of the irradiation affects the amount of germination, there is some evidence that the number of spores that die soon after germination is increased by increasing the intensity of the irradiation. If this early death is largely caused by the presence of chromosome aberrations which behave irregularly in mitosis, and if chromosome aberrations

show a time-intensity effect in *Neurospora* as in *Tradescantia* (Sax, 1940; Fabergé, 1940), such behavior is not unexpected.

Mutation rate in early- and late-germinating spores.—There was a possibility that mutant spores might differ in their time of germination or in their survival value in such a way that the proportions of mutants to normals picked out might vary according to whether early- or late-germinating spores were taken. To test this, spores isolated on the first day of germination were kept separate from those isolated on the second and third days of germination, and the mutation rates of the two groups were compared. The results obtained in three experiments are given in table 3. It is clear that there is no significant difference between the mutation rates of early- and late-germinating spores under the experimental conditions followed. This has considerable practical importance, as it shows that it is not necessary to isolate all the spores that germinate on a plate in order to get the mutation rate. The mutation rate can be estimated on samples of spores taken at any convenient time. From the theoretical point of view, it would appear that the occurrence of X-ray-induced mutation in a microconidium does not affect its germination.

Mutation rate at low and high intensity.—Table 4 gives the total numbers of survivors and mutants for a number of dosages from 2,250 r to 31,500 r given at an intensity of approximately 240 r per minute. The table is a summary of a number of different experiments, which proved to be homogeneous and are therefore considered together. Every individual experiment included a control and usually three X-ray series, so that if there had been any extensive variation it would have been possible

TABLE 3. Mutation rates in early- and late-germinating spores.

Dosage in r units	Experiment number	Isolated first day			Isolated second and third days		
		Survivors	Mutants		Survivors	Mutants	
			Number	Per cent		Number	Per cent
4,500	12	128	5	3.9	35	1	2.9
13,500	12	129	9	6.9	36	2	5.6
31,500	12	85	23	27.0	29	8	27.6
22,500	30	186	49	26.3	89	19	21.3
31,500	30	133	45	33.8	77	27	35.1
126,000	30	16	12	75.	44	34	77.3
9,000	26	65	4	6.2	100	7	7.0
18,000	26	145	13	9.0	112	14	12.5
31,500	26	127	32	25.2	113	25	22.1

to check whether an unusual value was limited to one dosage or was characteristic of the experiment. Every point was determined by more than one experiment. The mutation rate is about half that reported by Lindegren and Lindegren (1941). This discrepancy may be accounted for in two ways. In our experiments, only the easily distinguishable mutant types were selected, as it was thought that this would tend to keep the conditions of selection more uniform throughout the experiments. Secondly, by recording as mutants only those cultures that remained mutant at the end of two weeks we may have classed as normals some types that would have been included among the mutant by the Lindegrens.

TABLE 4. Effect of dosage on mutation rate at low intensity (240 roentgens per minute).

Dosage in r units	Survivors	Mutants	
		Number	Per cent
Control	810	5	0.6
2,250	723	20	2.8
4,500	421	21	5.0
9,000	677	42	6.2
13,500	592	44	7.4
18,000	640	68	10.6
22,500	204	34	16.7
31,500	325	80	24.6

It will be seen that the mutation rate increases with dosage in a linear manner, the highest dosage, 31,500 r, resulting in a mutation rate of 24.6 per cent.

Since it was found that the survival rate at 31,500 r was more than 50 per cent, and since in the case of ultraviolet irradiation an increase of energy leading to very low survival was accompanied by a drop in the mutation rate, it was decided to increase the X-ray dosage until a greater killing was observed, to see if this would be accompanied by a drop in the mutation rate among the survivors as in the ultraviolet experiments. Consequently, spores were irradiated at the Memorial Hospital, at an intensity of 5,400 r per minute, and the mutation results of these high-intensity treatments are given in table 5. The survival rate at 126,000 r was approximately

0.01 per cent, as given in table 2. However, at this dosage the mutation rate is still increasing, being 78.5 per cent as compared with 53.3 per cent at 63,000 r. Therefore, the X-ray data show no drop in mutation rate with a high death rate, comparable to that obtained as a result of ultraviolet irradiation.

TABLE 5. Effect of dosage on mutation rate at high intensity (5,400 roentgens per minute).

Dosage in r units	Survivors	Mutants	
		Number	Per cent
Control	527	1	0.2
15,750	657	94	14.3
22,500	274	68	24.8
31,500	772	243	31.5
63,000	60	32	53.3
126,000	65	51	78.5

The mutation rate in the high-intensity experiments increases linearly with dosage, as in the low-intensity experiments (see fig. 1). However, the mutation rate appears to be higher in the high-intensity experiments. Considering dosages of 22,500 r and 31,500 r, the mutation rate from the former was 16.7 per cent at low intensity and 24.8 per cent at high intensity, whereas from the latter it was 24.6 per cent at low intensity and 31.5 per cent at high intensity. These results are suggestive, but they had to be checked in experiments performed with the same X-ray apparatus before an intensity effect could be considered as definitely established.

Intensity effect.—Several experiments were made to test the presence or absence of the intensity effect. In two experiments a dose of 15,750 r, a continuous dose of 31,500 r, and a dose of 31,500 r given in two equal fractions separated by a gap of six hours were given. The intensity throughout was 5,400 r per minute. In both experiments the continuous dose of 31,500 r gave a higher percentage of mutations than the fractionated dose, and in one case this difference was significant (table 6). The fractionated dose, however, showed a rather small increase over the half dose of 15,750 r. It was thought possible, therefore, that the six-hour inter-

TABLE 6. *Effect of intensity and of fractionated treatment on mutation rate.*

Dosage in r units	Experi- ment	Sur- vivors	Mutants	
			Number	Per cent
Control	33	129	1	0.8
Control	34	74	0	0.0
Control	35	77	1	1.3
Total		280	2	0.7
15,750	33	270	39	14.4
(5,400 r per min.)	34	387	55	14.2
Total		657	94	14.3
15,750 + 15,750	33	207	46	22.2 \pm 2.89
(5,400 r per min.)	34	370	69	18.6 \pm 2.02
	35	328	80	24.4
Total		905	195	21.5
31,500				
(270 r per min.)	35	341	65	19.1 \pm 2.13
31,500	33	155	42	27.1 \pm 3.57
(5,400 r per min.)	34	286	88	30.8 \pm 2.73
	35	311	85	27.3 \pm 2.53
Total		752	215	28.6

val between the two treatments might have resulted in a change in the spores, such that they responded less readily to the second dose. In that case the higher mutation rate of the high-intensity experiments might not be due to an intensity effect but might result because the total dose was given earlier in the one case than in the other. Accordingly, another experiment (experiment 35) was made, in which the continuous dose was given at the end of the experiment. In this case a fractionated dose of 15,750 r plus 15,750 r, with an interval of about three hours, and two continuous doses of 31,500 r—one at an intensity of 270 r per minute, the other at an intensity of 5,400 r per minute—were given. The intensity was varied by varying the distance of the object from the source of the irradiation. In this experiment also, the high-intensity treatment gave the highest mutation rate, the difference between the rate at high and low intensity being significant. It seems, therefore, that there is a genuine increase in mutation rate with increase in intensity.

Correlation of visible mutants and sterility.—Lindegren and Lindegren (1941) observed a correlation between X-ray-induced visible mutants and the occurrence of partial sterility when the mutants were crossed to a standard line. They assumed that this sterility was due in the main to chromosomal aberration, and brought forward some evidence in support of this view. Since partial sterility has been shown to be usually correlated with chromosomal aberrations in other plants (C. R. Burnham, 1930; C. Pellew and E. R. Sansome, 1931), it was thought permissible to record sterilities and use them as an index of the occurrence of aberrations.

In order to determine the degree of fertility of a cross between a particular mutant and the standard

line, a number of ripe perithecia were pressed between two slides and the perithecial cases removed so that the clusters of asci were spread out flat on the slide. Completely sterile crosses were easily picked out, although these were always checked several times. The remainder were recorded for the number of asci having eight ripe spores and the number having one to seven ripe spores. When there

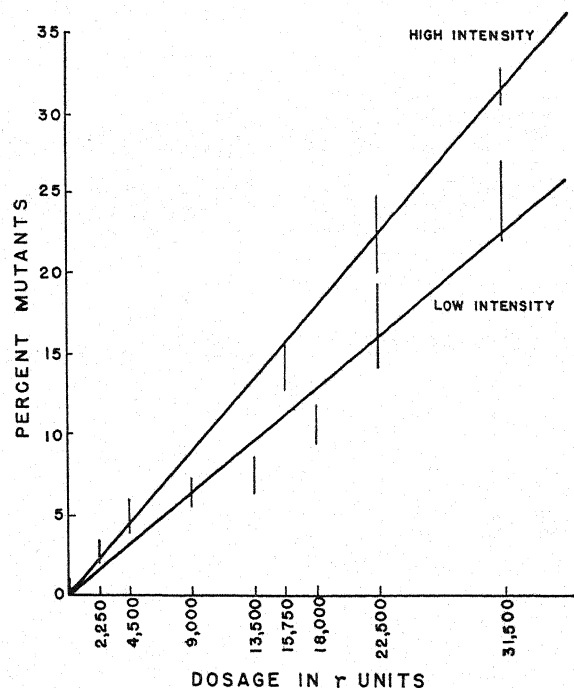


Fig. 1. Effect of dosage on mutation rate at low intensity (240 r per minute) and at high intensity (5,400 r per minute).

were 66 per cent or more of eight-spored asci, the cross was recorded as being as fertile as the controls, since the controls gave this result. When there were 50 per cent or less of eight-spored asci, the cross was recorded as sterile. Crosses having between 50 and 66 per cent of eight-spored asci were scored as questionable and were not included in the final results. They were infrequent. Since the occurrence of an ascospore lethal would lead to the failure of development of four of the ascospores without necessarily being associated with a chromosome aberration, cases in which no asci with more than four ascospores were found were given special consideration. They were infrequent, and in every case so far recorded, the number of asci with as many as four ripe spores was less than half the total number of asci recorded, so it was assumed that even if an ascospore lethal were present there was in addition sterility due to aberration. Such crosses, therefore, were classed among the steriles. Repeated records of the same cross gave similar results, and so the method of recording appears to be valid. There were a few cases in which different clusters of asci

TABLE 7. *Effect of dosage on production of visible mutants and sterility.*

Dosage in r units	Percentage		Mutants			Normals			Per cent mutants among	
	Mutants	Sterile	Total	Tested	% sterile	Total	Tested	% sterile	Fertiles	Steriles
9,000	6.7	23.8	11	11	54.5	154	37	21.6	4.0	15.3
18,000	10.5	31.5	27	23	56.5	230	21	28.6	6.7	18.8
31,500	23.7	32.3	57	28	78.6	183	28	17.9	7.5	57.8
15,750 ^a	14.2	34.1	55	48	77.1	332	137	30.7	5.6	29.4
22,500 ^a	24.8	45.8	68	54	70.1	206	53	37.7	13.5	38.1
31,500 ^a	32.3	62.0	129	107	89.7	278	178	48.3	8.4	46.3

^a High intensity (5,400 r per minute).

seemed to vary in their sterility. These were possibly heterokaryotic for a chromosome aberration. The crosses giving only empty perithecia present some difficulty, since they might be caused by gene mutation or by aberrations. However, when the part-steriles were classified into two groups, one with less than 25 per cent of complete asci, the other with 25 to 50 per cent of complete asci, it was found that increase of dosage or intensity led to an increase in the proportions of the more sterile class. The empty-perithecia class showed a similar behavior with increase in dosage or intensity. It was assumed, therefore, that most of the empty-perithecia types are due to complex aberrations; and such types have been included with the steriles.

It must be realized that this method of recording sterilities does not select all the aberrations that cause sterility. For example, in the case of a reciprocal translocation in which there is no crossing over between the centromere and the translocation point, the results of segregation are either complete disjunction or complete nondisjunction. This would be expected to lead, in the case of *Neurospora*, to the formation of eight-spored asci or asci with no viable spores. The latter type might be difficult to distinguish from unripe asci and from asci that have not developed for physiological reasons; and no attempt has been made to record them. Consequently, a class of aberrations which may be quite numerous has been left out of consideration.

The results are given in tables 7 and 8. The recording of sterilities is rather tedious, and therefore the data are not as extensive as would be desirable. The occurrence of part-steriles among con-

trols is infrequent; one was found among 134 cultures analyzed. In the case of the dosage of 9,000 r, the mutants to be recorded for sterility were taken from one experiment only; and since the mutation rate at this dosage is relatively low, the number of mutants to be recorded was low. Three classes in table 7 were irradiated at 5,400 r per minute; the remainder were irradiated at approximately 240 r per minute. When the results from the same dosage given at high and at low intensity are compared, it is seen that with high intensity there is an increase in the number of steriles as well as an increase in the number of visible mutants. Table 8 gives the results of two experiments made to test the intensity effect, in which sterility was also recorded. In the first experiment, in which a fractionated dose was compared with a continuous dose, the continuous dose was given at the beginning of the experiment. In the second experiment, the continuous dose was given at the end of the experiment. In both cases there was an increase in visible mutants and in the number of steriles from the continuous dose at high intensity.

The tables show that there is correlation between visible mutants and sterility such that the percentage of steriles among the visibles is about twice that among the phenotypic normals. This correlation may be somewhat reduced at high dosages and high intensity because of the greater increase in the number of steriles among the normals.

The correlation between visible mutants and sterility may be brought about in two ways. One type may be causally related to the other, as, for example, would be the case if position effects should

TABLE 8. *Effect of intensity on production of visible mutants and sterility.*

Dosage in r units	Percentage		Mutants			Normals			Per cent mutants among	
	Mutants	Sterile	Total	Tested	% sterile	Total	Tested	% sterile	Fertiles	Steriles
15,750 +										
15,750 H.I. ^a	18.6	37.6	69	57	84.2	301	136	31.6	4.9	37.9
31,500 H.I.	31.0	58.4	88	76	89.4	198	143	48.2	8.2	45.2
31,500 L.I. ^b	19.1	48.4	65	48	83.3	276	93	37.6	6.4	34.3
31,500 H.I.	27.3	73.6	85	51	86.3	226	43	67.4	13.7	32.5

^a H.I. = high intensity, 5,400 roentgens per minute.

^b L.I. = low intensity, 270 roentgens per minute.

occur or if duplications and deficiencies brought about by aberrations should appear as visible mutants. Alternatively, the same type of initial effect might lead to one or other or both of these end results. This situation would occur if single X-ray hits should induce chromosome breaks, and changes appearing as visible mutants should sometimes be associated with these breaks. Some of these breaks would unite with other breaks to give aberrations under suitable conditions, and in this way a correlation between aberrations and visible mutants would appear. There is evidence that some of the visible mutants result from aberrations. Thus it is found that the same dosage gives more visible mutants at high than at low intensity. This is associated with an increase in the number of steriles at high intensity. The number of initial effects is probably the same for the same dosage, whatever the intensity, and there is no reason to assume that a greater proportion of these would result in visible mutants at high than at low intensity. The most reasonable interpretation of the intensity effect is that some of the visible mutants are directly caused by aberrations. The question then arises whether or not all visible mutants are of this type. The fact that some visibles are recorded as fertile does not of itself settle the problem, since not all aberrations are identified by this method of recording sterility.

If all visible mutants were the result of aberrations, one would expect the percentage of visibles among the steriles to be the same, within certain limits, for all dosages. However, it will be seen that, whereas the percentage of visibles among the steriles is approximately the same for the same dosage given at different intensities or as a fractionated as opposed to a continuous dose (table 8), when the dosage is increased the percentage of visibles among the steriles also increases (table 7). This indicates that some of the visible mutants may be independent of aberrations. With regard to such changes, there are two main possibilities: the changes may be such that the visible mutants produced always result from potential breaks capable of being associated with aberrations; or, some of these visible mutants may be "gene mutations" and quite independent of breaks. In *Drosophila*, lethals have been found to be correlated with chromosome aberrations, whereas visible, apparently gene mutants seem to be independent of aberrations (Demerec, 1937). This suggests that there may be different types of X-ray effects, one (much the more frequent) causing chromosome breakage, the other involving a change in the gene without resulting in chromosome breakage (Muller, 1941). It was thought that perhaps the intensity experiments would distinguish between the two types. If some of the correlation between visible mutants and sterility occurred because the same initial change was capable of giving both visible mutants and aberrations, then at high intensity the same number of changes would be induced as at low intensity, but there would be a greater chance of these changes

being involved in aberrations. This would reduce the percentage of visible mutants among the fertiles at high intensity. If, however, the fertile mutants were due to changes independent of aberrations, the percentage of visible mutants among the fertiles would be the same irrespective of the intensity. Actually, the percentage of visibles among the fertiles is higher at high intensity and with a continuous rather than a fractionated dose. This may indicate that certain of the fertile visibles are due to undetected aberrations which increase with intensity.

Thus it was not possible in the present material to distinguish between one-hit effects associated with healed breaks and one-hit effects entirely independent of breaks.

SUMMARY

X-radiation produced variants in *Neurospora* which genetic analysis showed to be mutants. The increase in mutation rate with increase in energy followed a straight-line relationship at low dosages and reached 78.5 per cent at 126,000 roentgens.

Increase in intensity led to an increase in the mutation rate. Phenotypic normal and mutant cultures obtained from irradiated spores often showed partial sterility when crossed with a standard wild type, but this was more frequent in phenotypic mutants than in phenotypic normals. Partial sterility is believed to result from chromosomal aberration.

An analysis of the sterility and mutation rates at different dosages and intensities indicates that there are two types of mutants, one caused by chromosomal aberration and the other not. However, the latter class may consist partly or entirely of changes that are potentially capable of being associated with aberrations.

CARNEGIE INSTITUTION OF WASHINGTON,
COLD SPRING HARBOR, LONG ISLAND, NEW YORK, AND
INDUSTRIAL HYGIENE RESEARCH LABORATORY,
NATIONAL INSTITUTE OF HEALTH,
BETHESDA, MARYLAND

LITERATURE CITED

- BEADLE, G. W., AND V. L. COONRADT. 1944. Heterokaryosis in *Neurospora crassa*. *Genetics* 29: 291-308.
- BURNHAM, C. R. 1930. Genetical and cytological studies of semisterility and related phenomena in maize. *Proc. National Acad. Sci.* 16: 269-277.
- DEMEREK, M. 1937. Relationship between various chromosomal changes in *Drosophila melanogaster*. *Cytologia*, Fujii Jubilee Vol. 1125-1132.
- DODGE, B. O. 1930. Breeding albinistic strains of the *Monilia* bread mold. *Mycologia* 22: 9-38.
- . 1942. Heterokaryotic vigor in *Neurospora*. *Bull. Torrey Bot. Club* 69: 75-91.
- EMMONS, C. W., AND A. HOLLAENDER. 1939. The action of ultraviolet radiation on dermatophytes. II. Mutations induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. *Amer. Jour. Bot.* 26: 467-475.
- FABERGÉ, A. C. 1940. An experiment on chromosome fragmentation in *Tradescantia* by X-rays. *Jour. Gen.* 39: 229-248.

- HOLLAENDER, A., AND C. W. EMMONS. 1941. Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia on Quant. Biol. 9:179-186.
- , EVA R. SANSOME, E. ZIMMER, AND M. DEMEREC. 1945. Quantitative irradiation experiments with *Neurospora crassa*. II. Ultraviolet irradiation. Amer. Jour. Bot. 32:226-235.
- LINDEGREN, C. C., AND G. LINDEGREN. 1941. X-ray and ultra-violet induced mutations in *Neurospora*. I. X-ray mutations. Jour. Heredity 32:405-412.
- , AND —. 1941. X-ray and ultra-violet induced mutations in *Neurospora*. II. Ultra-violet mutations. Jour. Heredity 32:435-440.
- , 1942. The use of the fungi in modern genetical analysis. Iowa State College Jour. of Sci. 16:271-290.
- MULLER, H. J. 1941. Induced mutations in *Drosophila*. Cold Spring Harbor Symposia on Quant. Biol. 9:151-165.
- PELLEW, C., AND E. R. SANSOME. 1931. Genetical and cytological studies on the relations between Asiatic and European varieties of *Pisum sativum*. Jour. Gen. 25:25-54.
- SAX, K. 1940. An analysis of X-ray induced chromosomal aberrations in *Tradescantia*. Genetics 25:41-68.
- SHEAR, C. L., AND B. O. DODGE. 1920. Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. Jour. Agric. Res. 34:143-171.
- STADLER, L. J. 1932. On the genetic nature of induced mutations in plants. Proc. 6th Int. Congr. Gen. 1:274-294.
- , AND F. M. UBER. 1942. Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. Genetics 27:84-118.
- SWANSON, C. P. 1940. A comparison of chromosomal alterations induced by X-ray and ultra-violet radiation. Proc. National Acad. Sci. 26:366-373.

QUANTITATIVE IRRADIATION EXPERIMENTS WITH *NEUROSPORA CRASSA*. II. ULTRAVIOLET IRRADIATION¹

Alexander Hollaender, Eva R. Sansome, E. Zimmer, and M. Demerec

IT HAS been found that exposure to ultraviolet irradiation at sublethal doses will induce changes of a mutation-like character in *Trichophyton mentagrophytes* (Emmons and Hollaender, 1939; Hollaender and Emmons, 1941). These experiments were repeated with *Neurospora*, a fungus in which such changes could be subjected to genetical analysis. A similar study was conducted on the effects of X-rays on this organism. The results of X-ray treatment on mutation production are given in a separate paper (Sansome, Demerec, and Hollaender, 1945). In this report we will describe the effects of ultraviolet irradiation and discuss them in relation to the X-ray results.² Every effort was made to keep the conditions and techniques in the X-ray and the ultraviolet experiments as constant as possible, and the discussion of materials and all genetic and non-physical methods given in the first paper are applicable here.

ULTRAVIOLET IRRADIATION TECHNIQUE.—Microconidia were washed off with physiological salt solution, shaken thoroughly, filtered through absorbent cotton, and centrifuged in an angle centrifuge at about 4000 rotations per minute for 30 minutes. The precipitated spores were resuspended in physiological salt solution and shaken to break up any possible clumps. The approximate number of spores was determined in a Petroff-Hausser bacterial counting chamber, and usually was about 100,000 to 1,000,000 per cc.

¹ Received for publication December 23, 1944.

Appreciation is expressed to Mrs. M. B. Houlahan, who was connected with the earlier part of the study.

² The data on mutation, wavelength and dosage were obtained at the National Institute of Health. The second author accepts responsibility for the interpretation of results from the genetical aspect.

The radiation apparatus used in this study was substantially the same as that used in previous studies (Hollaender and Claus, 1936; Hollaender and Emmons, 1939; Emmons and Hollaender, 1939). The radiation of a medium-pressure water-cooled quartz capillary mercury vapor lamp of the Daniels-Heidt type was concentrated on the entrance slit of a quartz monochromator. The emerging monochromatic beam was concentrated on a standardized thermopile for the determination of the energy or the face of the exposure cell. All lenses, prisms, and windows were made of quartz with good transmission down to 2000 Å.

The material was exposed to the radiation in a cell which permitted the removal of 1/10-cc. samples during irradiation without interrupting the exposure. The material was stirred very thoroughly, to make certain that each spore received an equivalent amount of energy.

There are two ways in which to get a true picture of the amount of radiation each spore receives; one is to have a dense suspension which will absorb all the energy entering the exposure cell. The other method is to have a very dilute suspension in which practically all the energy entering the exposure cell is transmitted; i.e., the number of spores is so low that the amount of energy absorbed cannot be measured. In both techniques since the beam of ultraviolet does not cover the entire cell it is imperative that the material be stirred constantly, because it is often difficult to get a suspension of spores of high concentration. For this reason the method of irradiating very dilute suspensions was used. The amount of energy received by each cc. of spore suspension was determined for the incident energy at

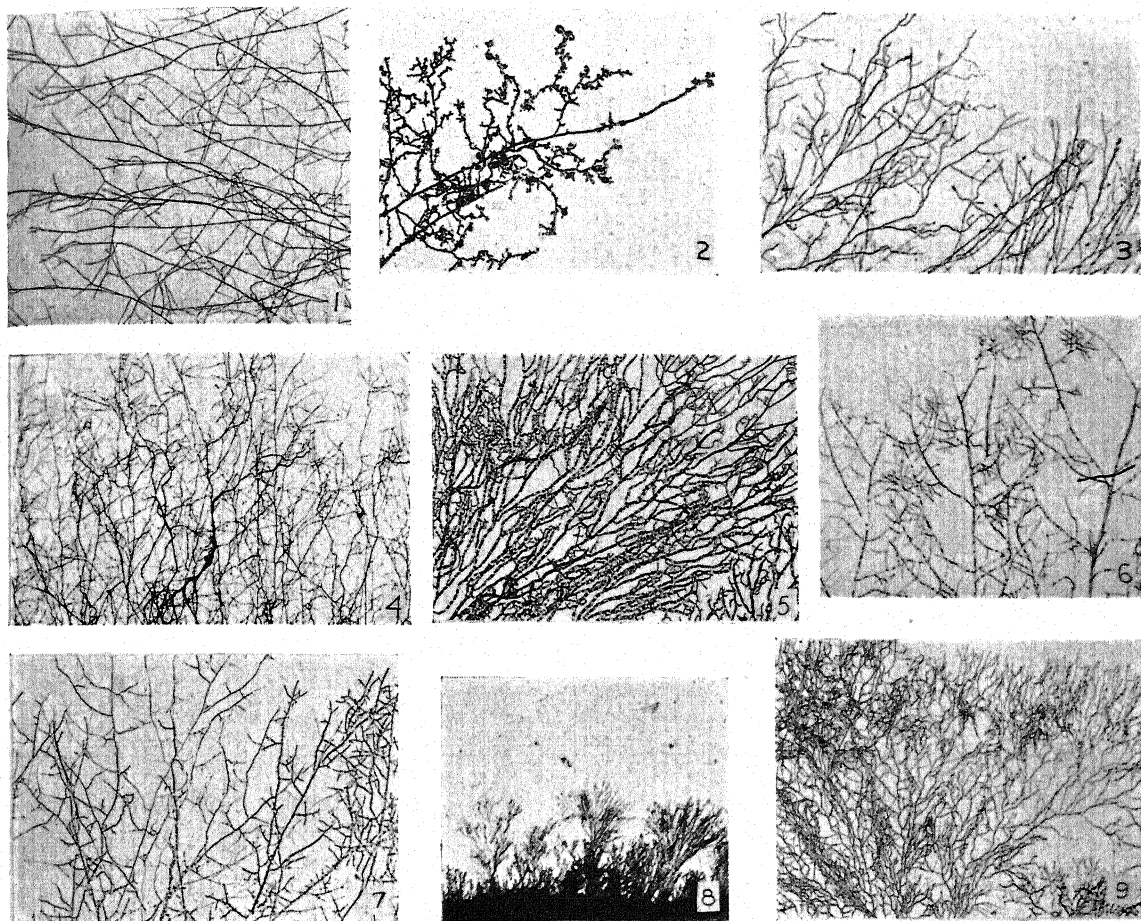


Fig. 1-9.—Fig. 1. Typical mycelium of *Neurospora crassa* (fluffy) coming from culture used as control.—Fig. 2 to 9. Different mutant types showing variation in branching and growth. All figures enlarged from original. The cultures were grown along a bare glass surface and photographed after 24 hour growth with exception of cultures of figures 2 and 9 which were photographed after 48 hours.

the exposure cell divided by the number of cc. irradiated. A typical calculation follows:

T = time of exposure in seconds.

I = energy per centimeter deflection of galvanometer in ergs per second.

d = centimeter deflection of galvanometer.

K = cubic centimeters per exposure cell.

$$\frac{d \times I \times T}{K} = \text{ergs per cc.}$$

The quantity that was removed for each exposure (1/10 cc.) was taken into account. A control sample was taken before each experiment was started. The control and experimental samples were diluted in physiological saline and plated out in different dilutions. The spores were isolated on germination and transferred to small tubes.

A method which was found very useful with other fungi (*Trichophyton mentagrophytes*, Hollaender and Emmons, 1939; *Aspergillus terreus*, Hollaender, Raper, and Coghill, 1945), namely, the determination of the survival rate after exposure to ultra-

violet, was not readily applicable to this study. *Neurospora crassa* has a spreading growth habit which makes it very difficult to determine the numbers that germinate on a particular plate. This difficulty is increased by the fact that the spores do not all germinate at the same time. Ultraviolet-irradiated spores show an even greater variability in time of germination than untreated spores. In general ultraviolet-irradiated spores grow more slowly at first and the extent of this slowing up of the growth rate is roughly a function of the energy which the spores had received.

TYPES OF MUTANTS RECORDED.—As in the X-ray experiments, cultures that were visibly different from normal were recorded as mutants. Certain of the cultures appeared mutant at first and later reverted to normal. When the reversion occurred in the first week, the cultures were classified as normal, but when it occurred later the cultures were recorded as mutants. Out of 303 mutants, 80 were colonial. The majority showed less than normal vigor. The grossly different appearance of the mu-

tants was usually correlated with a difference in branching habit as seen under the microscope. Figures 1 to 9 illustrate the branching habits of a few of the mutants found. A class of mutants that showed very reduced growth and usually either reverted to normal or died out on successive transfers was found. These mutants probably belong to the type called "degenerate phenotypes" by Lindegren and Lindegren (1941), and their occurrence seems

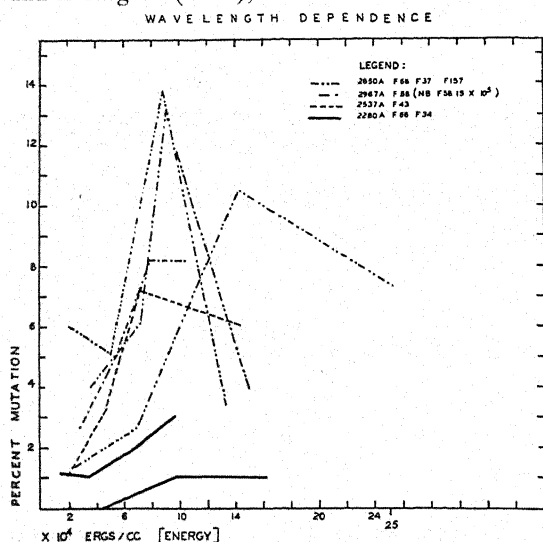


Fig. 10. Wavelength dependence of mutation production at 4 wavelengths. The energy values which will have to be multiplied by 10^6 for 2650, 2537, and 2280 should be multiplied by 10^5 for 2967.

to be a characteristic of ultraviolet irradiation. Out of 303 mutants, 46 which died out may be presumed to belong to this class. Some of the 68 reversions that occurred probably belong to this class also; but, as will be discussed later, reversion may be due to several causes.

WAVELENGTH DEPENDENCE.—A set of seven wavelengths, namely, 2280, 2380, 2480, 2537, 2650,

TABLE 1. Frequency of visible mutations produced by different wavelengths at various dosages.

Wave-length	Experi-ment	Energy in ergs/cc. $\times 10^4$	Number tested	Per cent mutation
2280 A	F 34	0	50	0
		4.44	100	0
		9.82	100	1
		16.18	100	1
	F 66	0	60	0
		1.36	191	1.1
		3.40	194	1
		6.81	205	2
		9.57	197	3
		0	53	0
2537 A	F 43	1.94	98	1
		4.38	97	3.1
		7.14	97	7.2
		14.32	83	6.0

TABLE 1. *Concluded.*

Wave-length	Experi-ment number	Energy in ergs/cc. $\times 10^4$	Number tested	Per cent mutation
2967 A	F 58	0	59	0
		28.4	81	2.6
		53.0	96	5.2
		78.1	72	8.2
		103.5	98	8.2
2650 A	F 37	0	51	0
		3.67	100	4
		7.12	100	6
		11.08	100	13
		14.99	100	4
	F 52	0	86	0
		3.79	106	.9
		7.17	100	1
		14.87	75	4
		19.97	96	7.2
	F 59	0	82	2
		7.65	102	5.9
		13.20	90	10
		19.30	100	5
		28.80	115	3.4
	F 68	0	64	0
		2.14	199	6
		4.96	198	5.1
		8.68	196	13.9
		13.58	183	3.3
	F 157	0	118	0
		2.31	240	1.7
		6.98	230	2.6
		14.27	229	10.5
		25.32	234	7.3
	F 17	0	108	0
		3.19	100	1
		7.13	100	0
		11.00	93	3.2
		16.52	91	1.1
	F 22	21.74	98	1
		40.64	99	4.0
		0	50	0
		6.80	311	1
		19.68	324	.3
	F 49	44.88	286	1.4
		84.94	292	3.8
		0	115	1
		5.03	111	1.8
		8.72	116	1.8
		12.92	83	7.2
		20.47	107	4.6

2805, and 2967 A were compared as to their effectiveness in mutation production. The work of Hollaender and Emmons (1941) demonstrates that the energy necessary for mutation production is lowest at 2650 A with a second minimum at 2280 and is highest at 2967. Because of these findings the regions of the ultraviolet spectrum illustrated in figure 10, namely 2280, 2650, and 2967, were emphasized in this study. For equivalent energy value, the killing rate was very high and the mutation rate

TABLE 2. Effects on survival of seven wavelengths at different energies.

Wave-length	Experiment number	Energy— ergs/cc. × 10 ⁴	Number ^a spores per cc.	Per cent survival
2280 A	F 34	0	325 × 10 ²	100
		4.4	163 × 10 ²	50
		9.8	71 × 10 ²	21.9
		16.7	15.5 × 10 ²	4.7
	F 42	0	84 × 10 ³	100
		3.8	127 × 10 ²	15.2
		6.5	42.3 × 10 ²	5.1
		9.1	73 × 10	.8
2380 A	F 45	15.1	43 × 10	.5
		0	72.6 × 10 ³	100
		2.4	52.5 × 10 ³	72.6
		7.2	120 × 10 ²	22.6
		16.6	59.6 × 10 ²	8.1
2480 A	F 46	33.7	109 × 10	1.5
		0	104.5 × 10 ³	100
		4.3	156.5 × 10 ²	15
		7.9	25 × 10 ²	2.5
		15.9	91 × 10	.8
2537 A	F 43	27.8	48.6 × 10	.4
		0	63 × 10 ³	100
		1.9	72.6 × 10 ³	+
		4.3	95.5 × 10 ²	15.3
		7.1	18 × 10 ²	2.3
2650 A	F 23	14.3	46 × 10	.7
		0	23.3 × 10 ³	100
		4.2	15 × 10 ³	35
		7.7	17 × 10 ²	10
	F 27	16.2	13 × 10	poor
		0	88.5 × 10 ²	100
		2.9	28.5 × 10 ²	29.9
		6.6	40.5 × 10	4.7
	F 37	13.4	37.5 × 10	4.4
		0	97.5 × 10 ³	100
		3.3	78 × 10 ³	67
		6.8	118 × 10 ²	14
	F 41	10.7	84.3 × 10	8.3
		14.7	84.5 × 10	.9
		0	179 × 10 ²	100
		2.5	60 × 10 ²	33.5
2805 A	F 44	4.9	61 × 10 ²	3.4
		8.3	36 × 10	2.0
		13.6	23 × 10	1.3
		0	80.5 × 10 ³	100
		5	50.2 × 10 ³	62.4
2967 A	F 40	8.3	12.6 × 10 ²	15.6
		14.5	36 × 10	4.5
		23.2	97 ?	1.2
		0	39 × 10 ³	100
		36	202 × 10 ²	52
		56.7	112 × 10 ²	28.8
		81.2	29 × 10 ²	7.4
		137.8	208 × 10	5.3

^a The first figure in column 4 gives the average of the counts on two to four plates multiplied by the dilution factor which then gives the number of colony-forming organisms per cc.

low at a wavelength of 2280; and at 2967 the killing and mutation rates were both low. It required a tenfold increase in energy at 2967 to cause killing and mutation rates of the order of magnitude given by 2650 A (tables 1 and 2).

MUTATION FREQUENCY AND DOSAGE.—Because there appears to be a certain variability in the response to ultraviolet treatment of spore suspensions coming from different cultures, the experiments cannot be averaged. Table 1 includes the results of eight experiments at a wavelength of 2650, the wavelength that was found to be most efficient in producing mutations. These experiments were selected from a number of others because they happened to have fairly high numbers of spores at each dosage. They are representative of the results obtained in other experiments. In the case of F37, F17, F22, and F41, fluffy + strains were used; and in the remaining experiments, fluffy — strains. It will be noticed that in five experiments the mutation rate increases with energy up to a certain level and then decreases with a further increase in energy.³ The peak of the curve occurs at approximately the same energy level, but its height varies from experiment to experiment. Experiment F32 does not show this behavior, nor do experiments F17 and F22. In the last two experiments the mutation rates are very low, and therefore the results are not very instructive. Moreover, in the case of F22 most of the runs involved very high energy values, at which values the maximum mutation rate would have been passed according to the results of the other experiments. The same strain was used in experiments F17 and F22, and it is possible that this was a strain with a low mutation rate. Such strains were also found in *Trichophyton* (Emmons and Hollaender, 1939).

THE FREQUENCY DOSAGE CURVE.—One explanation of a curve of this type, which is unique in irradiation experiments, is that the spore suspension is heterogeneous in respect to the amount of radiation absorbed by each spore or in the response to irradiation. The conditions of irradiation seem to preclude the idea of differential exposure of the individual spores. The possibility that the spores themselves are different in their response remains to be considered.

In measuring the mutation rate we are measuring the effect of the ultraviolet irradiation on the nucleus. However, there are two main ways in which the spores may be heterogeneous in respect to their mutational response to ultraviolet irradiation. The cell wall and the cytoplasm surrounding the nucleus may differ in their capacity to transmit the ultraviolet radiation at different stages, or the nucleus may give a different response at different stages of its cycle. One or both of these factors may be operative. Several experiments were made in the

³ Similar curves have been found in *Penicillium notatum* and *Aspergillus terreus* (Hollaender and Zimmer, 1945; Hollaender, Raper and Coghill, 1945).

TABLE 3. Frequency of mutations in young and old irradiated spores.

Experiment number	Young spores			Old spores		
	Energy	Number tested	Per cent mutation	Energy	Number tested	Per cent mutation
F 13	0	52	0	0	53	0
	7.30	154	4.5	6.46	148	3.3
	20.22	155	11.7	20.18	154	2.6
	43.42	145	10.6	45.37	129	1.6
	0	50	0	0	48	1
F 113	4.61	24	1	5.75	86	1
	14.67	88	5	15.45	89	3
	31.02	93	4	25.09	104	1
	0	50	0	0	47	0
	8.80	106	1.9	8.16	98	4.2
F 121	24.28	100	2.0	25.36	98	9.2
	49.74	106	1.9	48.26	108	4.6

hope of obtaining evidence regarding the heterogeneity of the treated spore samples.

IRRADIATION OF OLD AND YOUNG SPORES.—It was thought that if variations occurred, either in the capacity to absorb ultraviolet radiation or in the power of the nucleus to respond to the treatment, samples of predominantly young spores might differ from those of predominantly older spores in their mutation rate after ultraviolet irradiation. Consequently, comparable cultures were wetted down, and microspore suspensions prepared and irradiated after 24 hours and after 48 hours. Twenty-four hours after wetting, the number of microconidia was relatively low, and spores had to be taken from 15–20 cultures in order to obtain suspensions of a desirable density. The results of three experiments involving young and old conidia are given in table 3. These results are insufficient; but it will be seen that, whereas in experiments F113 and F13 the mutation rates of the young conidia were higher, in experiment F121 the reverse occurred. Thus, while the data do not exclude the possibility of a different mutational reaction of young and old conidia to ultraviolet irradiation, they afford no evidence of any consistent effect of that kind.

ANALYSIS OF NORMAL PHENOTYPES IN RELATION TO CHROMATID EFFECTS.—Stadler and his associates found that the endosperms from crosses involving ultraviolet-treated pollen of *Zea Mays* showed a great number of fractional deficiencies (Stadler and Uber, 1942). They suggest that these deficiencies are explicable if only one chromatid derived from a treated chromosome is affected, the other being normal. It is desirable to consider the results of such chromatid effects in *Neurospora*. If, as an effect of ultraviolet irradiation, one of the two nuclei resulting from the first nuclear division after treatment should be normal, and the other mutant, the normal nucleus might be expected to predominate over the mutant so that the resulting culture would appear normal. In *Neurospora*, therefore, chromatid effects would be masked, and only chro-

mosome effects would be visible. The following simple hypothesis to explain the rise and subsequent fall in the mutation rate was considered. It was assumed that chromatid effects in general are not visible, only chromosome effects appearing as visible mutants. It was further assumed that the type of effect, whether chromatid or chromosome, is conditioned largely by the nuclear stage of the microconidium undergoing treatment, only microconidia at a certain stage being capable of full chromosome effects. On these assumptions, the mutation rate would rise with dosage until every conidium had received approximately one effect. At this point those microconidia that were capable only of a chromatid response would appear phenotypically normal, since the mutant would be covered by the normal allele. Of the microconidia that gave a chromosome response to the ultraviolet treatment, those undergoing lethal mutations would be eliminated and the remainder would appear as visible mutants. An increase in dosage leading to coincident mutations would result in a reduction in the number of visible mutants in the class of microconidia giving chromosome effects, because of the coincidence of lethal and visible mutations. The extent of the reduction of mutation rate would depend upon the relative proportions of visible and lethal mutations. In the case of microconidia giving chromatid effects only, the occurrence of two mutations in one nucleus would be followed by the separation of the two mutants into one nucleus in half the cases, when the remaining normal nucleus would be expected to predominate. In the other half of the cases the two mutations would pass to different nuclei and might be expected to produce a balanced heterokaryon. In experimentally made heterokaryons between pairs of visible mutants, it was found that nine out of fourteen combinations appeared normal. Hence a high proportion of balanced heterokaryons might be expected to appear normal eventually, although they might not achieve a balance at first, in which case they would first appear mutant and later would appear normal.

It was thought, therefore, that the microconidia giving only chromatid responses to ultraviolet irradiation would continue to appear normal even when two or more mutations were present, owing to the balanced heterokaryon phenomenon, whereas the number of visible mutants from the microconidia giving chromosome effects would decrease because of the coincidence of lethal and visible mutations in a certain proportion of cases. This would account very well for the dosage-mutation curve actually found. On this hypothesis, a fair proportion of phenotypically normal cultures from heavily treated microconidia would be expected to be balanced heterokaryons. Accordingly, a microconidial analysis of a number of these normals was made. Subcultures about one week old were wetted down, and the microconidia so obtained were plated out and later isolated into tubes. Out of twenty cultures from a spore sample given a dosage of 17.9×10^4 ergs per cubic centimeter at 2650 (experiment F12, run 5), one was a balanced heterokaryon consisting of two semi-lethal mutants, four gave one mutant from approximately 30 isolated microconidia, and fifteen gave only standard fluffy cultures. Out of 16 cultures given a dosage of 21.7×10^4 ergs per cc. (experiment F17, run 5), all gave only fluffy cultures; and out of 13 cultures given a dosage of 40.6×10^4 ergs per cc. (experiment F17, run 6), only fluffy cultures were obtained in all cases except one, where one mutant was obtained. Thus, out of 49 cultures tested, only one was a balanced heterokaryon. Five were possibly heterokaryotic for normal and a mutant character; but, since the microconidia came from subcultures taken from old cultures, these mutants may have been due to spontaneous mutation and not to chromatid mutation during irradiation. As will be seen later, some of the reversions were probably balanced heterokaryons; but, since they appeared mutant at first and

TABLE 4.

Class	Number of cultures	Types recovered
A	4	Fluffy only
B	4	Dwarf or scant at first; later fluffy
C	5	Mutant only
D	11	Mutant and fluffy
E	11	More than one type of mutant

were so considered when the data for the dosage-mutation tables were collected, they cannot be considered as helping to keep up the proportion of normal phenotypes at high dosages. The results on the microconidial analysis of normal phenotypes recovered from high dosages, therefore, seem to eliminate the hypothesis that the drop in the dosage-mutation curve is due to heterogeneity of microconidia with respect to their capacity to give a chromosome or chromatid response to ultraviolet irradiation.

REVERSIONS AND CHROMATID EFFECTS.—As mentioned before, a number of cultures that were mutant at first later reverted. The general nature of unstable forms is discussed in the X-ray paper. Out of 303 mutants, 68 reverted sooner or later. No correlation could be found between the frequency of reversion and the wavelength or dosage. A microconidial analysis of 35 reverted mutants gave the results summarized in table 4.

The mutants falling into class A may be of three types: (1) Genuine reverse mutations, in which the "fluffy" nuclei have overgrown the original mutant type. In this case it should be possible to recover mutants from crosses involving the original culture before it reverted. (2) A type described by the Lindgrens as "degenerate phenotype," which gives only wild-type and fluffy progeny when crossed with a standard wild-type line. Such types most probably result from an effect of the ultraviolet radiation on the cytoplasm. (3) Normals wrongly classified as mutants.

Class B seems to consist of a special type of mutant which is slow-growing at first but attains normal vigor after growing for a time. Ascospores from mutants of this class appear dwarf or scanty at first and normal later.

In the case of class C, in which only mutants were recovered, three explanations are possible. (1) The phenotypic reversion might be due to a gene mutation to normal fluffy, but the original mutant nuclei might be still so numerous that a small spore sample would detect only mutants. (2) The culture on growing might acquire a cytoplasmic adaptation to the mutant gene which is lost when single conidial cultures are taken. This might well be a more extreme example of the type of phenomenon shown by class B mutants. (3) The third possibility is that the original culture consisted of a balanced heterokaryon between a lethal mutation and a visible mutation. On microconidial analysis, only the visible mutants would be viable and therefore recoverable. In this case we must assume that two chromatid changes occurred at the time of irradiation, one to a visible mutation, the other to a lethal, and that these were distributed to different nuclei at the first nuclear division. The culture appeared mutant at first, but after a favorable ratio had been established between the two types of nuclei it appeared normal.

Class E, in which more than one type of mutant was obtained, evidently consists of balanced heterokaryons involving two visible mutants. Class D consists of heterokaryons between fluffy and a fluffy mutant type. It is difficult to know whether these types were heterokaryotic from the time of irradiation or whether they were entirely mutant at first and became heterokaryotic by mutation. Each particular mutant would have to be investigated carefully, to show which is the more probable explanation.

However, in the case of class E, and probably of class C also, we have fairly clear evidence of a

chromatid rather than a chromosome effect of the irradiation. Since these cases involve coincidental chromatid changes, it would seem that chromatid changes are quite frequent, as might have been expected from Stadler's results on maize. Moreover, it may well be that some of the non-reverted visible mutants are heterokaryons.

Analysis of balanced heterokaryons should afford a method for estimating the relative proportions of visible and lethal mutations. The present data, in which there were 11 balanced heterokaryons involving two visible mutations (class E) and 5 involving possibly one visible and one lethal (class C), might indicate that lethals are less frequent than visibles; but a more extensive analysis is needed to settle this question.

THE OCCURRENCE OF MULTIPLE MUTATIONS.—In view of the possibility that the spore samples were heterogeneous in their response to ultraviolet radiation, it was thought advisable to determine, if possible, the proportion of coincidental mutations among the ultraviolet-induced mutants. Accordingly, ascospores from crosses involving some of the fluffy mutants and the wild-type were grown, and the progeny recorded. In crosses involving twenty-eight mutants, whole asci were dissected and the ascospores removed in linear order; in the case of forty other mutants, ascospores from the cross with wild-type were sown at random. The results from the single-ascus dissections were: thirteen cases in which one mutant type was recovered together with fluffy and wild-type; eight cases in which no mutant was recovered, although more than four spores germinated; three cases in which no mutant type was recovered, but only four spores or less germinated; and four cases in which more than one mutant type was recovered. If the eight types in which no mutant was recovered belong to the degenerate-phenotype class described by Lindegren and are not gene mutations, then out of twenty genetic mutants four involve two or more genetic loci. From the forty mutants subjected to random ascospore analysis, more than one mutant type was recovered nineteen times, and only one mutant type twenty-one times. The discrepancy between the results from whole-ascus dissections and those from random ascospore sowing may be partly due to the fact that for some crosses very few asci were dissected, so that a second mutant type might have been present and not recovered. In any case, the numbers are small and the difference is not significant. There is no doubt that the finding of twenty-four mutants with two or more mutational changes out of sixty investigated indicates a very high degree of coincidence of mutations. No correlation could be detected between dosage and the occurrence of coincidental mutations.

STERILITY.—Since visible mutations were found to be correlated with sterility in the case of the X-ray-induced mutants, a number of the ultraviolet-induced mutants were investigated for sterility by the technique used in analyzing the X-ray mutants.

Of forty-seven mutants so examined, forty were as fertile as the controls, three were partly sterile, two had empty perithecia, and two were doubtful part-steriles. It is difficult to make a close comparison between the X-ray and ultraviolet results, because the ultraviolet mutants were from samples that had been subjected to different amounts of radiation and that gave different mutation rates. However, in the X-ray cultures, even at the lowest dosage examined, about 50 per cent of the mutants were partly sterile. Thus there is a distinct difference in frequency of sterility in the ultraviolet- and X-ray-induced mutants, although ultraviolet-induced mutants may sometimes be sterile.

DISCUSSION.—In a study of the effects of seven wavelengths between 2200 and 3000 Å, three were especially studied—namely, 2650, 2280, and 2967 Å. At 2650, nucleic acids have high absorption; and this wavelength is most efficient in producing killing and mutation effects; that is, lower amounts of energy are required to produce a given effect at this wavelength than at the others. At 2280, nucleic acids, proteins, and other constituents of the cell—including the cell wall—absorb the radiation rather intensely, as is shown by photographs taken at this wavelength (Cole, 1941). Therefore it is likely that at 2280 most of the radiation to which the cell is exposed will be absorbed by the cell wall and the cytoplasm. This might be expected to kill the cell rather than produce changes in the nucleus, and this is what actually seems to occur. At 2967, very little measurable absorption is found; but with very high dosages both killing and mutational effects can be obtained.

The typical ultraviolet dosage-mutation curve rises to a maximum, and with a further increase of dosage the percentage of mutations decreases. There is a certain variability in mutation rate among spore samples. A genetical analysis of the mutants obtained shows that the proportion of multiple mutations is high. A possible explanation of the dosage-mutation curve, based on the assumption that only a certain proportion of the spores can give a full chromosome response to ultraviolet irradiation, the remainder giving only chromatid changes, was tested and not verified. However, the possibility that the spores differ in the capacity of their nuclei to respond to ultraviolet irradiation, some being at a stage at which no mutation can occur, is not excluded. The high proportion of multiple mutations is in accordance with such a hypothesis. Some of the results of Stadler are interesting in this connection (Stadler, 1939). He found that ultraviolet-irradiated pollen produced deficiencies in the endosperm much more frequently than in the embryo, whereas X-rayed maize pollen yielded a high frequency of deficiencies in both endosperm and embryo. Among seeds which gave only five A deficiencies in the F_1 plants, there were more than a hundred complete A deficiencies in the endosperm. Stadler suggests several possible explanations for this phenomenon, of which the third—namely, that

"there is a difference in some secondary effect after fertilization, for the course and rate of early development are very different in endosperm and embryo"—may be considered here. It may be that ultraviolet treatment causes an effect upon the gene of such a nature that if nuclear division follows very rapidly after the treatment the gene is unable to reproduce itself and a full or fractional deficiency results. However, if division is delayed there may be a possibility of recovery in many cases, so that the number of mutations obtained is greatly decreased. It may be that the effects on the *Neurospora* spores resemble the embryo in that there is a greater chance of recovery than in the endosperm. In order to explain the *Neurospora* ultraviolet data on this basis, we would assume that the irreversible changes would occur more readily in spores at a certain stage. There is some evidence in the work of Swanson (1942) that the response to ultraviolet irradiation is affected by the stage of the nucleus. He treated nuclei in the pollen tubes of *Tradescantia* at various times after sowing on an agar plate and found the number of chromatid breaks to be decreased when the dose was applied at a later than an earlier stage.

An alternative explanation is based on the dual action of the ultraviolet irradiation rather than on heterogeneity among the spores. On this assumption the ultraviolet is believed to have a direct effect on the cytoplasm, apart from its effect in inducing genetic mutations. This effect on the cytoplasm increases with dosage in a cumulative fashion. An interaction takes place between the nucleus and cytoplasm such that mutant nuclei have a smaller chance of surviving in a defective cytoplasm than do normal nuclei. The differential survival value of the normal and mutant nuclei is increased with increased dosage, and this results in a final drop in the mutation rate. In support of this, we have the occurrence of degenerate phenotypes, which show no evidence of being the result of genetic changes but appear rather to be due to cytoplasmic effects. In the case of *Trichophyton*, Hollaender and Emmons (1941) were enabled by suitable treatment of spores after irradiation to increase the mutation rate at higher dosages. This might be due to recovery of the cytoplasm, leading to increased survival of mutated nuclei. The supposition that mutants have an increasingly lower survival rate than normal phenotypes, however, is not in accord with the high frequency of multiple mutations. To account for this, one would have to assume that different mutations differ in respect to their effect on survival, and that deleterious mutations are more frequent at higher dosages. This might be accounted for if the reaction between mutants and cytoplasm is specific; that is, if certain mutants can only survive when certain specific elements in the cytoplasm are uninjured.

COMPARISON OF X-RAY AND ULTRAVIOLET RESULTS.—Because there is no direct method of comparing X-ray and ultraviolet dosage, comparisons between

them must be based on biological effects. One such effect is the killing rate of the treatment. The highest mutation rate recorded in the ultraviolet-treated material was 13 per cent, with about 9 per cent survival, whereas with X-ray treatment such a mutation rate would be associated with about 70 per cent survival. There is no doubt, therefore, that X-rays are more effective than ultraviolet irradiation in inducing mutations, when considered in relation to their killing effect. This is in line with the results of Lindegren and Lindegren. They found that less than 1 per cent of the ultraviolet-treated spores survived, and that, of the survivors, about 9 per cent were mutants; whereas in the case of X-rays, about 50 per cent of the treated spores survived, and of these about 25 per cent were mutants. Moreover, it was found that, with ultraviolet irradiation, increase in dosage led to a decrease in the mutation rate, associated with a rapid increase in the killing rate. In the case of X-ray treatment, the number of mutants increased with dosage even up to a dosage of 126,000 r, when only 0.01 per cent of the spores survived the treatment but 78.5 per cent of these were mutants.

The X-ray mutants differed from the ultraviolet mutants in that they were often associated with sterility, whereas ultraviolet mutants were generally fertile. If sterility is usually due to chromosomal aberration, as seems probable, then its frequent occurrence in the X-ray-treated material and its rare occurrence in the ultraviolet-treated material is in accordance with the results of Stadler and his collaborators in maize, and also with the work on *Drosophila*—especially that of Mackenzie and Muller (1940), Demerec, Houlahan, and Hollaender (1942), and Slizynski (1942). The X-ray results indicate that there are at least two types of change leading to the production of visible mutants. The intensity effect seems to indicate that some mutants are directly due to aberrations; whereas the fact that the percentage of mutants among the steriles is not constant, but increases with dosage, indicates that there are some mutants not directly due to aberrations. The data do not enable us to distinguish between mutants that are independent of aberrations ("gene mutations") and mutants that do not result from aberrations but may be associated with them (potential breaks). The rate of occurrence of mutations not resulting from aberrations might afford a more valid basis for comparison with the ultraviolet results than the total mutation rate. Unfortunately, the fact that all aberrations cannot be detected by the present methods makes such a comparison impossible. There is no doubt that the rate of occurrence of such mutations would increase more slowly with dosage than the total mutation rate.

A special type of change, called "degenerate phenotype" by Lindegren and Lindegren, merits further consideration. Degenerate phenotypes either die out or revert to normal on sub-culture, and when crossed with wild-type they give only fluffy and wild-

type progeny. They are therefore thought to be due to an effect on the cytoplasm rather than to a genetic effect. Their frequency is much higher following ultraviolet than following X-ray treatment: the Lindgrens found twelve such types among 36 ultraviolet-induced variants, and one out of 20 among X-ray-induced variants; and we found 40 out of 119 ultraviolet-induced variants, and three out of 99 X-ray-induced variants of this type. This high frequency among the ultra-violet-induced variants is believed to indicate that ultraviolet radiation has a much greater effect upon the cytoplasm than X-radiation, and the high death rate of the ultraviolet-treated spores may be due primarily to the effect of the treatment on the cytoplasm rather than on the nucleus. Any discussion of the possible mode of action of ultraviolet and X-radiation must still be of a highly speculative character. Because of the high absorption coefficient of nucleic acid in the ultraviolet, especially at 2650, it seems probable that the mutational changes produced by ultraviolet are largely caused by initial effects on the nucleic acid. Thus, as was pointed out by Mackenzie and Muller (1940), the ultraviolet radiation is selective in its action on the chromosomes. In contrast to this, the X-rays are not absorbed differentially, but they seem able to cause chromosome breakage wherever a sufficiently large cluster of ionizations occurs (Lea and Catcheside, 1942). It has been shown by Swanson (1942) that ultraviolet radiation will produce chromatid but not chromosome breaks in the pollen-tube chromosomes of *Tradescantia*. What relationship such breaks bear to the visible mutants produced by ultraviolet in *Neurospora* is not known. The characteristic mutation curve shown by ultraviolet-treated spores is most simply explained by heterogeneity of some sort. This is confirmed by the high degree of occurrence of multiple mutants. The simplest hypothesis to explain this would be that not all the microconidia are uninucleate, a certain proportion of them being binucleate. This explanation, however, seems to be precluded by the X-ray results, where there is no evidence of any such heterogeneity. If the heterogeneity resides in the nuclear condition, then certain nuclear stages must be less susceptible to ultraviolet treatment than others, as suggested earlier. If there are certain spores with nuclei that are more responsive to the action of ultraviolet radiation than those of other

spores, the question arises as to what happens to these spores when they are subjected to X-radiation. It may well be that such spores give a higher proportion of changes of the more localized type assumed to result from ultraviolet treatment, and if we could distinguish these changes from the other types induced by X-radiation we might obtain a curve for such mutants similar to the ultraviolet dosage-mutation curve.

SUMMARY

The wavelength most effective in inducing mutations in *Neurospora* was 2650. At 2280, the killing rate was high and the mutation rates were low, whereas at 2967 both killing and mutation rates were low unless very high dosages were given.

At 2650, the mutation rate increased with dosage up to a maximum and then decreased as in the case of *Trichophyton*.

Genetic analysis showed 24 out of 60 mutants to be multiple mutants. The rate dosage curve and the high coincidence of mutations are believed to indicate heterogeneity in the treatment given to the spores and in the response of the spore to treatment.

The hypothesis that spores differ in their capacity to give a chromosome rather than a chromatid response to ultraviolet radiation was found insufficient to explain the data, although evidence that both chromatid and chromosome effects occur was obtained.

The occurrence of sterility in association with the mutants was much less frequent in the case of the ultraviolet-induced mutants than in the X-ray-induced mutants.

The ultraviolet results are discussed in relation to the X-ray results and it is suggested that the ultraviolet effects are localized, whereas the X-ray effects are more diversified, including chromosome breaks and rearrangements. The X-ray effects may include effects of the type induced by ultraviolet treatment but this cannot as yet be determined since it is not possible to distinguish between the different types of mutants.

INDUSTRIAL HYGIENE RESEARCH LABORATORY,
NATIONAL INSTITUTE OF HEALTH,
BETHESDA, MARYLAND
CARNEGIE INSTITUTION OF WASHINGTON,
COLD SPRING HARBOR, NEW YORK

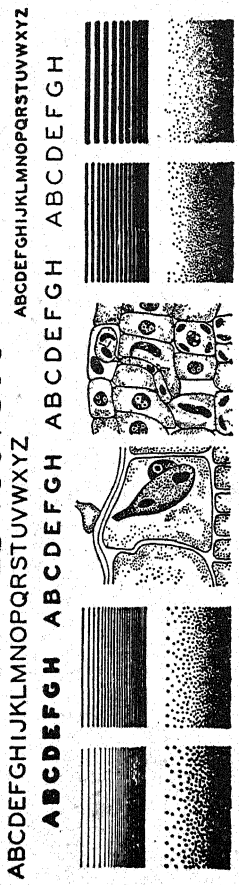
LITERATURE CITED

- COLE, P. A. 1941. The ultraviolet absorption spectra of different regions of *Trichophyton mentagrophytes* spores. Amer. Jour. Bot. 28: 931-934.
- DEMEREK, M., A. HOLLAENDER, AND M. B. HOULAHAN. 1942. Effect of monochromatic ultra-violet radiation on *Drosophila melanogaster*. Genetics 27: 139-140.
- EMMONS, C. W., AND A. HOLLAENDER. 1939. The action of ultraviolet radiation on dermatophytes. II. Mutations induced in cultures of dermatophytes by exposure of spores to monochromatic ultraviolet radiation. Amer. Jour. Bot. 26: 467-475.
- HOLLAENDER, A., AND W. D. CLAUS. 1936. The bactericidal effect of ultraviolet radiation on *Escherichia coli* in liquid suspensions. Jour. Gen. Physiol. 19: 753-765.
- , AND C. W. EMMONS. 1939. The action of ultraviolet radiation on dermatophytes. I. The fungicidal effect of monochromatic ultraviolet radiation on the spores of *Trichophyton mentagrophytes*. Jour. Cell. and Comp. Physiol. 13: 391-402.
- , AND C. W. EMMONS. 1941. Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symposia on Quant. Biol. 9: 179-186.

- , K. B. RAPER, AND R. D. COGHILL. 1945. The production and characterization of ultraviolet induced mutations in *Aspergillus terreus*. I. Production of the mutations. Amer. Jour. Bot. 32:160-165.
- , AND E. M. ZIMMER. 1945. The effect of ultraviolet radiation and X-rays on mutation production in *Penicillium notatum*. (Abstract) Genetics 30:8.
- LEA, D. E., AND D. G. CATCHESIDE. 1942. The mechanism of the induction radiation of chromosome aberrations in *Tradescantia*. Jour. Genetics 44:216-245.
- LINDEGREN, C. C., AND G. LINDEGREN. 1941. X-ray and ultraviolet induced mutations in *Neurospora*. I. X-ray mutations. Jour. Heredity 32:405-412.
- , AND ———. 1941. X-ray and ultraviolet induced mutations in *Neurospora*. II. Ultraviolet mutations. Jour. Heredity 32:435-440.
- MACKENZIE, K., AND H. J. MULLER. 1940. Mutation effects of ultraviolet light in *Drosophila*. Proc. Roy. Soc. London B 129:491-517.
- SANSOME, E. R., M. DEMEREC, AND A. HOLLAENDER. 1945. Quantitative irradiation experiments with *Neurospora crassa*. I. X-radiation. Amer. Jour. Bot. 32:218-226.
- SLIZYNSKI, B. M. 1942. Deficiency effect of ultraviolet light on *Drosophila melanogaster*. Proc. Roy. Soc. Edin. 61 B:297-315.
- STADLER, L. J. 1939. Genetic studies with ultraviolet radiation. Proc. 7th International Genetical Congress:269-275.
- , AND G. F. SPRAGUE. 1936. Genetic effects of ultraviolet radiation in maize. I. Unfiltered radiation. II. Filtered radiation. III. Effects of nearly monochromatic 2537 and comparison of effects of X-ray and ultraviolet treatment. Proc. Nat. Acad. Sci. 22:572-591.
- , AND F. M. UBER. 1942. Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. Genetics 27:84-118.
- SWANSON, C. P. 1942. The effects of ultraviolet and X-ray treatment on the pollen tube chromosomes of *Tradescantia*. Genetics 27:491-503.



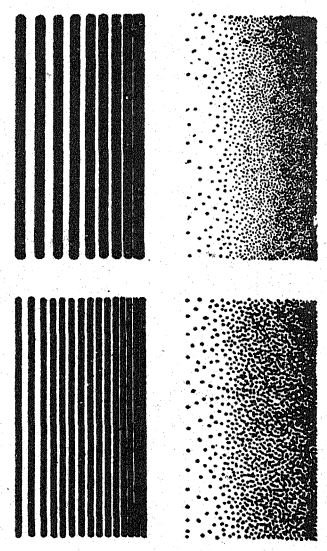
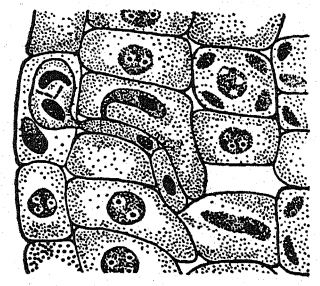
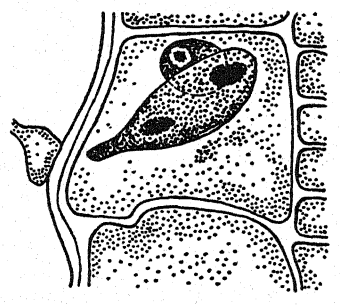
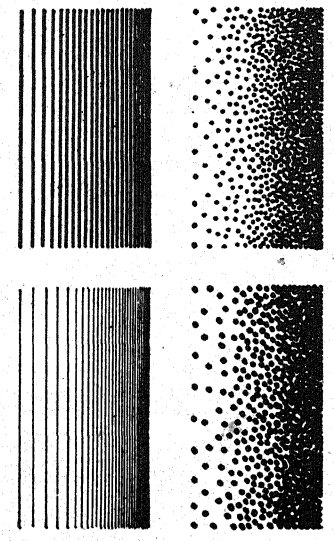
ABCDEFGHIJKLMNOPQRSTUVWXYZ
 1234567890



ABCDEFGHIJKLMNOPQRSTUVWXYZ
 1234567890

ABCDEFGHIJKLMNOPQRSTUVWXYZ

ABCDEFGHI ABCDEFGH



DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

Reprinted by permission of the authors from: RIKER, A. J., and REGINA S. RIKER. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

METASTATIC (GRAFT) TUMORS OF BACTERIA-FREE CROWN-GALLS ON VINCA ROSEA¹

Philip R. White

BRAUN (1943) HAS shown that crown-galls of *Vinca rosea* can be freed of the inciting organism by thermal treatment according to the methods developed by Kunkel (1941), without altering the capacity of the tissues to produce typical galls at the loci of inoculation. The same author had shown earlier (Braun, 1941) that similar bacteria-free crown-galls occur on *Helianthus annuus*, destruction of the bacteria resulting from unknown natural causes. Morphologically similar bacteria-free galls of genetic origin occur spontaneously on certain hybrid *Nicotianas* (Kostoff, 1930). White has reported the isolation of rapid-growing autonomous tissue cultures from *Nicotiana* tumors (1939) and from *Helianthus* galls (White and Braun, 1942) and has shown that both types of tissue culture are capable of producing tumors in new hosts by grafting (White, 1944; White and Braun, 1942). Since we now have transplantable tumors originating as a result of (1) genetic causes, and (2) infectious causes but freed of the infection by unknown natural means, it seemed desirable to determine if these galls of *Vinca* might not represent a third type of transplantable tumor, in which one step in its production, the process of eliminating the infection, is brought about experimentally under controlled conditions. The fourth, and most desirable type of all, would, of course, be one in which all causal processes involved in gall inception are of known and controllable nature. Braun and Laskaris (1942) have made one additional step in this direction in isolating the effect of naphthalene-acetic acid and other "growth substances" in supplementing the gall-inducing effects of attenuated strains of bacteria.

MATERIALS AND METHODS.—Through the courtesy of Dr. Armin C. Braun bacteria-free tumors of *Vinca rosea* were obtained having the following origin (Braun, 1943). Young healthy plants of *Vinca rosea* were inoculated by the multiple needle puncture method with a 24-hour nutrient dextrose-broth culture of the highly virulent Brown Peach strain of *Phytomonas tumefaciens*. The plants were allowed to stand on a greenhouse bench for five days during which time the infection was established and the tumefacient process presumably went to completion. The plants were then transferred to a hot room at a temperature of 46–47°C. for a period of five days, a period sufficient to destroy the bacteria effectively. The plants were then returned to the greenhouse at a temperature of about 25°C., and allowed about ten weeks time for the development of mature galls of

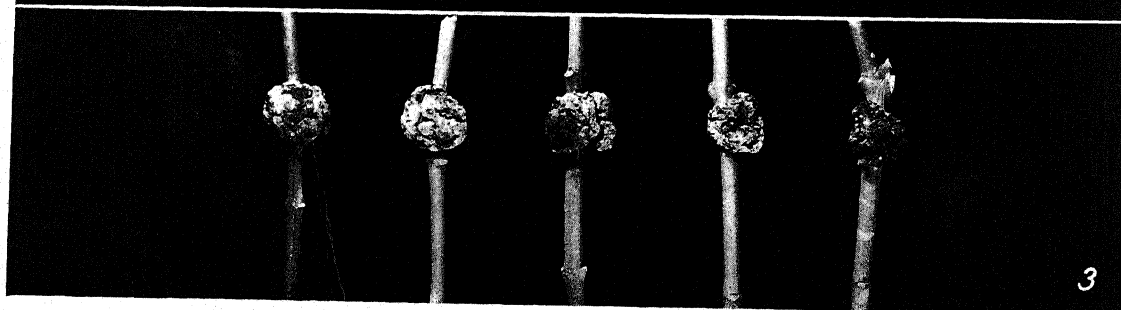
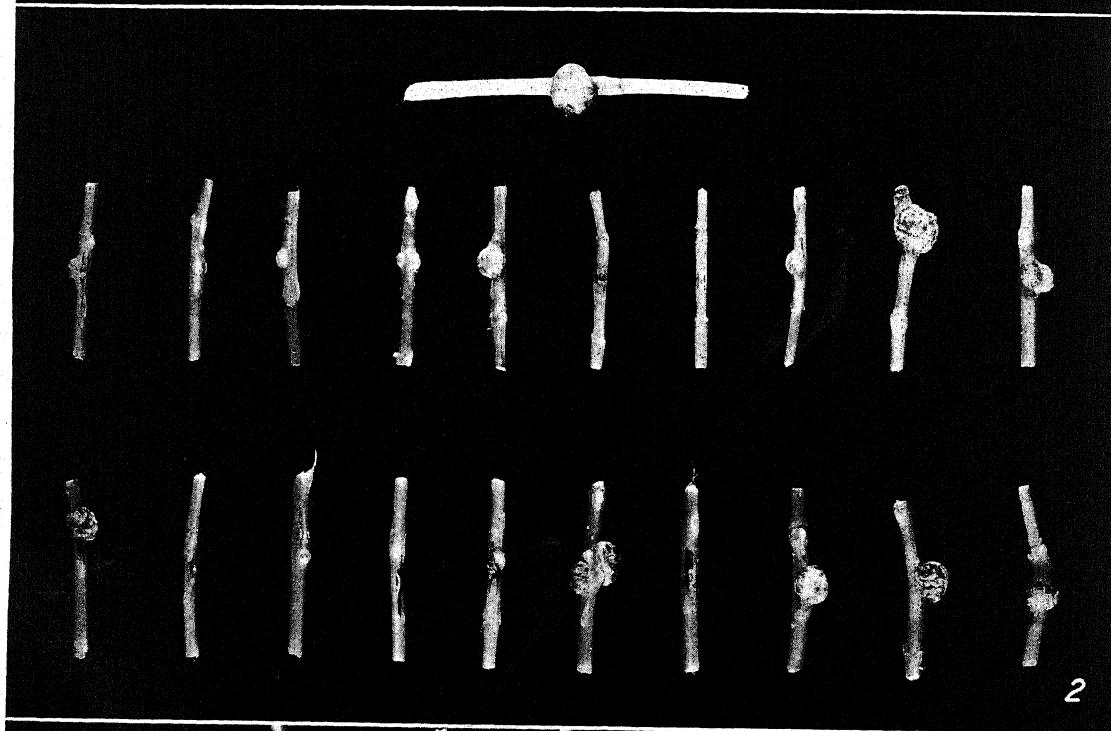
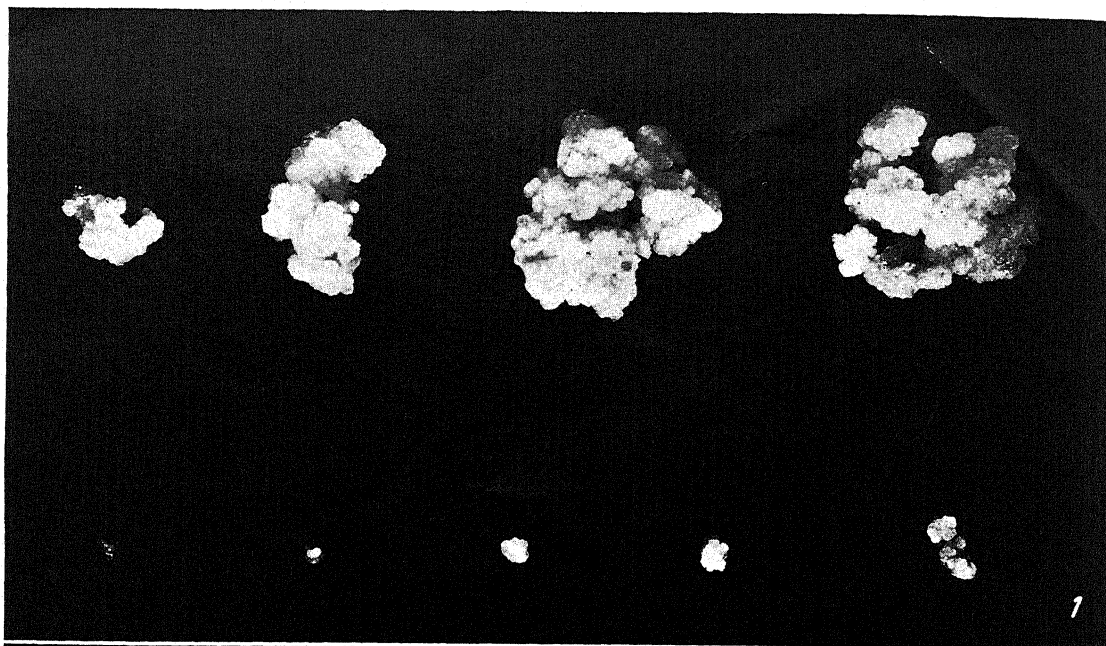
the type illustrated in Braun's 1943 paper. These steps were carried out by Dr. Braun and the tumors given to me at this stage.

Mature galls were broken open carefully so as not to contaminate the inner tissues with surface-borne molds or bacteria. Surface disinfection was not used. Fragments of the softer, more rapidly growing tissues were removed with a sterile 1.0 mm. surgeon's ear curette and placed on a semi-solid medium made up of White's nutrient (1944) stiffened with 0.6 per cent of thoroughly washed agar.

EXPERIMENTAL RESULTS.—Eighty isolations were made on April 13, 1943. Fifty-seven of these developed contaminations, mostly molds, but with some yellow bacteria. All isolations showing bacterial contaminations were re-inoculated by Dr. Braun into young healthy tomato plants. In no case did crown galls develop, showing that the bacteria present were not *Phytomonas tumefaciens* but random contaminants. Eighteen of the twenty-three aseptic cultures were transferred to fresh nutrient on April 20, the other five being discarded. On May 4 one more was discarded, four were divided, each into two cultures, and twelve were again transferred without division. Subsequently the derivatives of all cultures except No. 19 were discarded although many grew moderately well. No. 19, however, gave the most satisfactory results and by the eighteenth passage (December 13, 1943) a clone of twenty-five rapidly growing cultures had been built up from this one isolation. Individual cultures of the sixteenth to twenty-fifth passage (passage 20 excepted) are shown in figure 1. Since passages, with the exception of the first one, were of two weeks duration this picture, taken April 12, 1944, represents cultures at the time of transfer (lower row, left) up to eighteen weeks old (upper right). The very rapid rate of increase, as compared to that of *Nicotiana* or *Helianthus* cultures, is evident. These cultures are brilliant white in color and even at an age of four months without transfer show little or none of the brown discoloration and necrosis so characteristic of cultures of *Nicotiana* (White, 1939) or *Helianthus* (White and Braun, 1942) at the same age.

The microscopic structure is similar to that of other tissue cultures (White, 1939; White and Braun, 1942) but differs in some significant respects (fig. 5). The general texture, as a corollary of the rapid growth, is uniformly open showing neither the central core of radial organization of the *Nicotiana* tumor cultures first studied, with its well defined meristematic centers (White, 1939),

¹ Received for publication January 5, 1945.



nor the solidly organized whorls of scalariform elements (White, 1943; White and Braun, 1942) and marginal meristematic regions of the *Helianthus* cultures. There are many giant cells. Some of these may represent primitive latex chambers but have not been definitely so identified. Scalariform cells do occur in considerable numbers but are scattered. The general pattern appears completely without organization. It is therefore all the more surprising to find, as is shown in figure 6, a series of five pairs of primary xylem elements arranged in a perfect arc suggesting the early organization of a normal stem yet bedded in a parenchyma without any other suggestion of organization. The factors responsible for the even spacing and arcuate arrangement of these isolated elements certainly present interesting possibilities for study.

At the end of the twentieth passage (39 weeks after isolation) fragments of seventeen cultures were grafted into young healthy plants of *Vinca rosea* by the double incision method described elsewhere (White, 1944). Of this first series four (24 per cent) formed tumors. The host plants were at this time rather young and a second series set up March 1 gave better results. Forty-two grafts were made, eighteen from material of passage nineteen, and twenty-four from material of passage twenty. Of these only nine (21 per cent) failed to form tumors, thirty-three (79 per cent) being positive. The tumors formed were of clean, smooth, fleshy texture, up to 20 mm. in diameter (fig. 2, 3). The structure of these tumors and of the tumor-host connection (fig. 4, 7) shows no significant differences from those described elsewhere for bacteria-free galls of sunflower and *Nicotiana* (White, 1944).

Discussion.—Here, then, is still another type of bacteria-free tumor of plants possessing the properties of malignancy, metastasis (by grafting) and autonomous, discoordinate development. The process of tumefaction, the fundamental change in cell response, goes to completion in a very short time, probably a matter of a few hours, after exposure to the tumefacient agent (Braun, 1943; White and Braun, 1943). Once this change has been consummated the tissues are able to go on to produce small tumors *in situ*, after destruction of the inciting agent (Braun, 1943). Cultivation *in vitro* has resulted in the segregation of a particularly active type of tissue capable of producing massive tumors upon transplantation into healthy host plants. The large size of tumors produced by grafts in comparison with those commonly induced in comparable regions of the host plant by multiple needle puncture with a bacterial inoculum (compare

Braun, 1943, fig. 1 C) raises certain questions as to possible causes. The first possibility that comes to mind is that of better nutrition. Tumors produced by needle puncture inoculation have a very small initial point of contact with the host. The neoplastic cells, however, arise directly from the host so that the vascular connection develops without break in continuity simultaneously with the development of the tumor. Its only source of morphological inefficiency should be the deranged growth processes themselves. Such tumors, however, are usually present in large numbers as a result of the customary multiple inoculation and may be subject to considerable nutritional competition. Graft tumors are usually made singly or at least widely spaced on the stem, hence not subject to severe competition. We believe that they arise, however, from the implant and not from host cells (White and Braun, 1942). An entirely new vascular connection must therefore be established between two traumatized surfaces. One might expect such a connection to suffer in morphological efficiency from its developmental discontinuity, yet the tumors produced by such a process regularly exceed in size those produced by multiple bacterial inoculation (compare figs. 2 and 3 with Braun, 1943, fig. 1 C). Still larger tumors can be induced by decapitating young stems and smearing a bacterial inoculum over the cut surfaces. Here the vascular connection develops continuously as in the small needle puncture tumors yet has a broad anatomical base (the entire ring of vascular bundles) and is free from competition as in the graft tumors. These galls sometimes reach the size of a hen's egg. A nutritional explanation of these differences is reconcilable with the known morphological facts only if we assume the developmental continuity or discontinuity to result in insignificant anatomical differences and the factor of competition to be of crucial importance. A second possibility is that the presence of bacteria along with the tumor cells may reduce the vitality of the host tissues to a point where their residual growth capacity will support only a minimal level of tumor development. If this were the case one would expect that thermal inactivation of the bacteria would result in an increase in the size of tumors produced at the site of inoculation. Such an increase does not occur so that this explanation appears untenable. A third possibility, against which there are no such obvious cogent objections, yet in favor of which we as yet lack adequate evidence, involves the rather obscure property of "virulence." It is well known that certain mammalian neoplasia, which are at first difficult to transplant and when grafted produce

Fig. 1-3.—Fig. 1. Cultures of tissues from tumors of *Vinca rosea* rendered bacteria-free by heat therapy. Bottom row, left, as transferred, thereafter 2, 4, 6, and 8 weeks old. Top row, 12, 14, 16, and 18 weeks. The older cultures are glistening white and quite friable. $\times 1$. (Photograph by J. A. Carlile.)—Fig. 2. Tumors formed on healthy plants of *Vinca rosea* as a result of implantation of tissue cultures like those shown in figure 1. Implants were from passage 20. Out of twenty-one grafts only 3 (No. 6 and 7 from the left, top row; No. 2, bottom row) failed to "take." Photograph taken six weeks after implantation. $\times 0.65$. (Photograph by J. A. Carlile.)—Fig. 3. Older grafts, twenty-eight weeks after implantation. These tumors show considerable dead tissue on the surface and some necrosis. (Photograph by J. A. Carlile.)

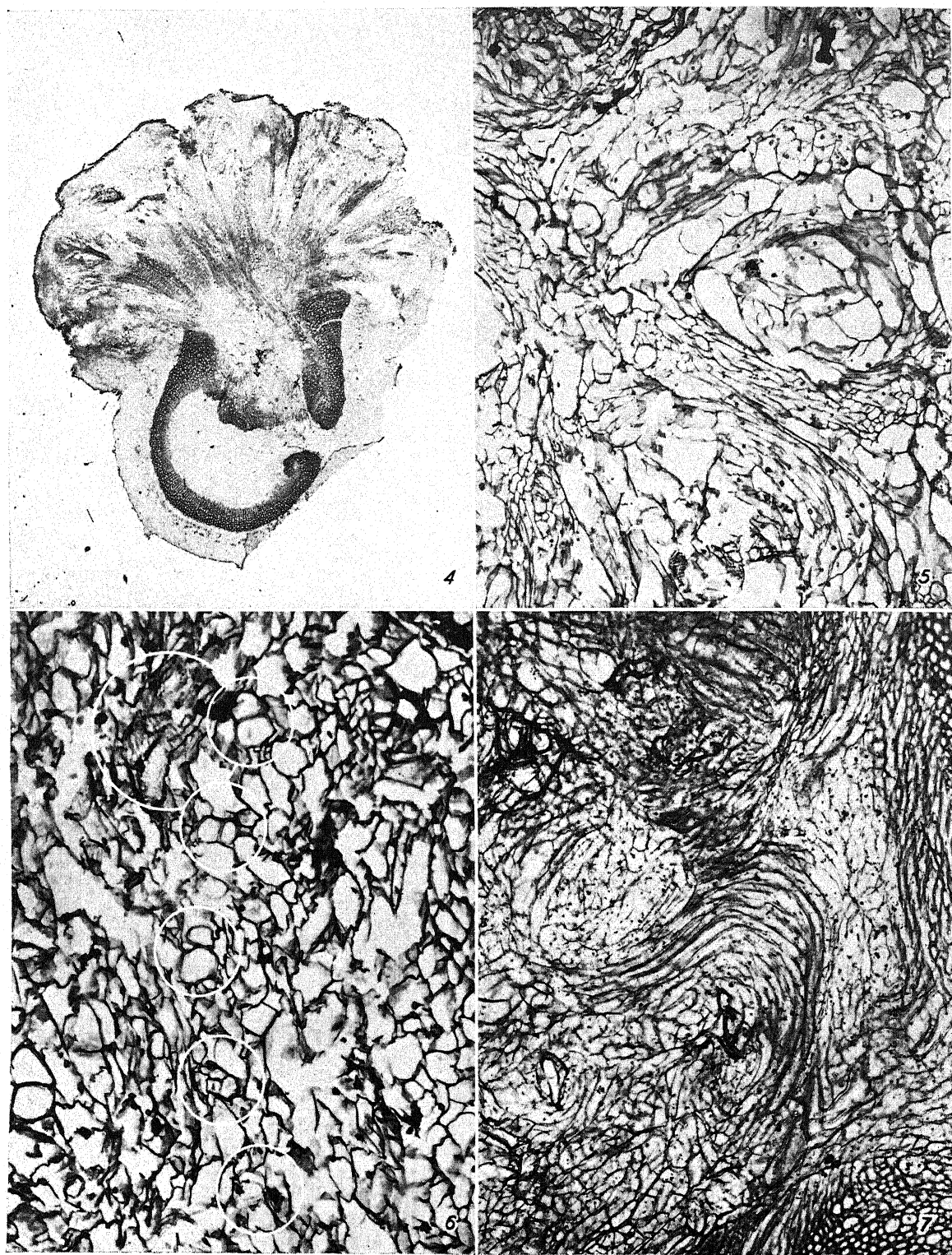


Fig. 4-7.—Fig. 4. Transverse section (free-hand) through a graft tumor on *Vinca rosea* (fig. 2, bottom row, second from the right). The incision at the right has healed normally without closure of the vascular ring. The other, parallel incision has been invaded by the developing tumor. The cortical flap between the incisions (upper right) has produced woody tissue on its inner as well as outer face and woody xylem has also formed in the pith facing the tumor. The location of the original implant is marked by an area of gummy necrosis at the center of the picture. $\times 13$. (Photo-

small and relatively benign tumors, undergo a change upon repeated passage through fresh hosts or after prolonged cultivation *in vitro* as a result of which their "virulence"—their capacity to produce tumors on transplantation and the size, persistence and malignancy of those tumors—is increased. It is possible that a similar result of *in vitro* cultivation may have taken place in the periwinkle tumors considered in this paper, and that the very high percentage of "takes" upon transplantation as compared to the results of multiple bacterial inoculation may represent a *real increase* in virulence of the tumor tissues involved. If substantiated this explanation might provide a new point of attack for study of the tumefacient property itself. If there is a real increase in virulence under *in vitro* conditions one might reasonably hope to determine those conditional variants capable of bringing about or preventing such an increase and thus learn the nature of the property itself. There may, of course, be still other explanations which do not immediately suggest themselves.

One other consideration suggested by this work is quite unrelated to the primary problem of tumefaction. *Vinca rosea* belongs to the Apocynaceae which are noteworthy among herbaceous plants for their production of latex. Some of the large cells of tissue cultures obtained from these tumors look very much as if they might be latex cells or

channels. The cultures are remarkable for their very rapid growth and the absence of apparent necrosis even in very large tissue masses. It seems possible that they might prove useful as materials in which to study, *in vitro* and under controlled conditions, the mechanisms of latex production. In view of the current importance of rubber production problems of all sorts this might be a useful subject for investigation although it is not likely to have the permanent importance that attaches to the investigation of tumefaction mechanisms.

SUMMARY

Tissue cultures were isolated from crown-galls on *Vinca rosea* rendered bacteria-free by heat therapy. These show a very rapid discoordinate growth *in vitro* and when grafted back into healthy host plants produce typical tumors which regularly exceed in size those generally produced by direct multiple needle puncture inoculation of tumefacient bacteria. Possible implications of this increase in growth capacity of bacteria-free over bacteria-containing tumors are discussed. The possibility of using these cultures in the study of latex and rubber production is also suggested.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY

LITERATURE CITED

- BRAUN, A. C. 1941. Development of secondary tumors and tumor strands in the crown gall of sunflowers. *Phytopathology* 31:135-149.
- . 1943. Studies on tumor inception in the crown-gall disease. *Amer. Jour. Bot.* 30:674-677.
- , AND T. LASKARIS. 1942. Tumor formation by attenuated crown-gall bacteria in the presence of growth-promoting substances. *Proc. National Acad. Sci. (U.S.A.)* 28:468-477.
- KOSTOFF, D. 1930. Tumors and other malformations on certain *Nicotiana* hybrids. *Zentralbl. Bakt. Parasit. Infekt., Abt. II.* 81:244-260.
- KUNKEL, L. O. 1941. Heat cure of aster yellows in periwinkles. *Amer. Jour. Bot.* 28:761-769.
- WHITE, P. R. 1939. Potentially unlimited growth of excised plant callus in an artificial nutrient. *Amer. Jour. Bot.* 26:59-64.
- . 1943. A handbook of plant tissue culture. The Jaques Cattell Press, Lancaster, Pa. 277 pp.
- . 1944. Transplantation of plant tumors of genetic origin. *Cancer Research* 4:791-794.
- , AND A. C. BRAUN. 1942. A cancerous neoplasm of plants. Autonomous bacteria-free crown-gall tissue. *Cancer Research* 2:597-617.
- , AND ———. 1943. Some evidence on the etiology of cancerous properties as exemplified in plant cells. (Abstract) *Jour. Bact.* 46:109-110.

graph by J. A. Carlile.)—Fig. 5. Transverse section through a tissue culture (fig. 1) showing the loose, open texture, characteristic of these cultures. A single scalariform element is visible at the bottom of the picture. There is no evident organization and no clearly distinct meristematic centers are visible although the culture was growing rapidly when fixed. $\times 127$. (Photograph by J. A. Carlile.)—Fig. 6. Transverse section of tissue culture showing five evenly spaced and arcuately arranged pairs of scalariform elements in cross section, and at the upper left a sixth group in longitudinal section, bedded in a loose, irregular parenchyma. $\times 250$. (Photograph by J. A. Carlile.)—Fig. 7. Transverse section of the region of origin of a graft tumor taken from the center of a section similar to that shown in figure 4. Gummy necrotic areas, presumably remnants of the original implant, are visible at the center and upper left hand corner of the picture. Normal host xylem at the right hand upper and lower corners. The resemblance between this tissue, developed under the mechanical strains present in the growing host stem and that grown *in vitro* where external stresses are at a minimum (fig. 5) is striking. $\times 127$. (Photograph by J. A. Carlile.)

THE EXTRACTION OF AUXIN FROM TOMATO FRUIT¹

Wesley P. Judkins

A NUMBER of methods have been reported by which auxin may be extracted from plant tissues: Went (1928), Thimann (1934), Laibach and Meyer (1935), Boysen Jensen (1936), Linser (1939), Avery, Creighton, and Shalucha (1940), Skoog and Thimann (1940), Thimann and Skoog (1940), Avery, Berger, and Shalucha (1941), Link, Eggers, and Moulton (1941), and Avery, Berger, and White (1944). The extraction of auxin from green plant tissue has been particularly difficult and no entirely satisfactory method is available at present. The tests reported in this paper were conducted in an attempt to secure further information on extraction procedures in preparation for work on certain horticultural problems associated with pollination and fertilization (syngamy) of flowers and growth of fruit.

MATERIALS AND METHODS.—Skoog's (1937) "de-seeded" *Avena* test method was used for auxin assays. All yields are expressed in terms of "Total Degrees Curvature per gram of fresh tissue (TDC/-gm)," following the formula suggested by Avery, Creighton, and Shalucha (1941). Except for a few tests cited in tables 6 and 8, all tomatoes used in these experiments were of the Stone variety. All extracts were tested at three dilutions to be sure auxin concentrations in the agar blocks were within the proportionality range. All yield figures in the tables or text are the average of at least duplicate racks of twelve *Avena* test plants.

An attempt was made from the start to follow a semi-micro technique, since a method was desired which could be used with small samples of flower parts and very small fruit. The samples used in these tests varied from 0.5 to 2.0 grams fresh weight. Although these are not micro quantities, they represent smaller samples than those which are commonly reported in the literature.

The drying of organic solvent extracts.—Organic solvent extracts of auxins are frequently dried in flasks from which the residue is recovered by adding water or agar sol. Since the evaporating solvent splashes over the inside of the flask, complete recovery of the dried residue is difficult or impossible.

In order to place the procedure on a somewhat more accurate semi-micro basis, a rather simple technique for evaporating liquid from organic extracts was developed. Figure 1 illustrates the apparatus used in this procedure. A small Pyrex test tube 100 × 13 mm. is fitted with a rubber stopper through which are inserted two glass tubes. Tube A is connected to a suction line and causes air to flow in

through B across the surface of the liquid in the tube. The tube may be immersed in a water bath of the desired temperature, depending on the solvent being evaporated. The first few milliliters of solvent are placed in the tube before the rubber stopper and air tubes are inserted. As the solvent evaporates, more may be added through tube B until the entire sample is dry and the residue is left concentrated at the bottom of the tube. This residue is recovered by adding agar sol and stirring thoroughly with a short glass rod.

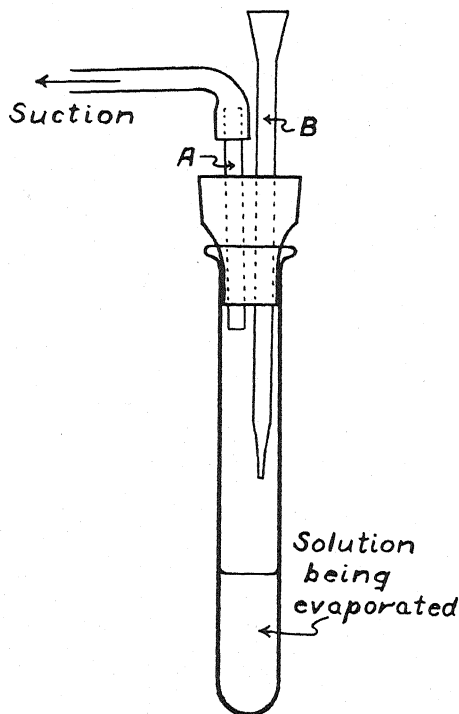


Fig. 1. Test tube with air ducts for the rapid drying of organic solvent extracts.

The use of organic solvents in extracting auxin from green tomato fruit.—After considerable exploratory work the following general extraction procedure was adopted. The fruit tissue was cut into 1/8-inch cubes. Samples of 2.0 grams were carefully weighed out and placed in a test tube to which 15.0 ml. of solvent were added. At the end of the extraction period, the solvent was poured off and the sample rinsed twice with 5.0 ml. of fresh solvent. This extract was then evaporated according to the procedure outlined in the previous section. Since the tomato tissue contained 94.0 to 95.0 per cent water there were frequently from 0.5 to 2.0 ml. of water extract left in the tube after the more volatile ether, alcohol, or acetone had been evaporated. In such cases 3.0 per cent agar was used to give a final agar block concentration of 1.5 per cent for the *Avena*

¹ Received for publication January 13, 1945.

The author wishes to express his thanks to Dr. F. S. Howlett, Ohio Agricultural Experiment Station, who suggested this problem, and Dr. G. S. Avery, Jr., Brooklyn Botanic Garden, for counsel and assistance during the course of this investigation. The work was conducted at the Ohio Agricultural Experiment Station, the University of Connecticut, and Connecticut College.

TABLE 1. *The use of organic solvents in extracting auxin from green tomato fruit. Extraction from 2.0 grams of fresh carpel-wall tissue of green tomatoes 4 cm. in diameter, picked from vigorous plants in early and mid-July. Samples cut into 1/8-inch cubes.*

Solvent and treatment of samples	Auxin yield during extraction periods			Total auxin yield TDC/g.
	1st 18 hrs.	2nd 2 days	3rd 2 days	
Acetone at 5°C.....	430	275	290	995
Acetone at 26°C.....	430	240	250	920
Acetone with ethanol rinse at 26°C.....	120	800	400	1320
Acetone with methanol rinse at 26°C.....	200	250	140	590
Dioxane at 26°C.....	0	0	0	0
Ethyl acetate at 26°C.....	150	0	0	150
Methanol at 26°C.....	0	0	0	0
Methanol with acetone rinse at 26°C.....	0	130	...	130
Methanol with ethanol rinse at 26°C.....	0	800	270	1070
Ether at 5°C.....	150	0	0	150
Ether at 26°C.....	350	0	0	350
Ether at 26°C.....	250	0	0	250
Ethanol at 26°C.....	850	600	505	1955
Ethanol at 70°C., 4 15-minute periods.....	1050
Ethanol with methanol rinse at 26°C.....	900	640	300	1840
Ethanol at 26°C. Auxin yield from one sample in a series of extractions, each of 24-hour duration: (1) 800, (2) 600, (3) 1000, (4) 550, (5) 700, (6) 674, (7) 200.....				4525

test. When samples were evaporated to dryness, 1.5 per cent agar was used in preparation for the *Avena* test.

Many different extractions were made of tomato carpel-wall tissue, and the most typical and significant results are reported in table 1. The earliest tests were carried out at 5°C. as suggested by van Overbeek (1938). It was found, however, that higher yields could usually be obtained if the extraction took place at 26°C. Temperatures of 70°C. or 100°C. did not increase the yield of auxin and usually reduced it.

Table 1 shows that dioxane or methanol alone are not suitable as solvents for auxin extraction from tomato carpel-wall tissue. Ethyl acetate gave yields of approximately 150 to 350 TDC per gram; acetone, 900 to 1000; and ethanol, 1000 to 4500. Whenever ethanol was used as a rinse after acetone or methanol, the yield was increased over that for acetone or methanol alone. This, as well as the definite superiority of ethanol over all other solvents, indicates that under the conditions of the present test, ethanol is superior to ether, acetone, methanol, dioxane, or ethyl acetate as a solvent for extracting auxin from fresh tomato carpel-wall tissue.

With the exception of ether, the tests of organic solvents reported here bear out the observations of other workers that several extractions are needed to remove all of the hormone from plant tissue. Gustafson (1939) reported on the extraction of auxin with ether from several fruits such as tomato, pepper, cucumber, and bean. His yields from tomato carpel wall varied from 36 to 400 TDC per gram, with the two highest being 257 to 400. If variations

in sampling technique, extraction procedure, variety and season are taken into consideration, the yields compare fairly well with the 250 and 350 TDC per gram reported in table 1. One extraction by Gustafson (1939) from carpel-wall tissue of a tomato fruit from a plant low in vigor in the winter yielded only 2.0 TDC per gram.

The superiority of alcohol over ether in the present tests is not in agreement with the reported results of several other investigators who used other plant tissues. Although no conclusive evidence is presented to explain this condition, two suggestions are offered. It has been pointed out by Thimann and Skoog (1940) and by Avery, Creighton, and Shalucha (1940) that the same extraction methods are not equally effective on different plant tissues. A comparison of various organic solvents for extracting auxin from tomato tissue has not previously been reported in the literature. It is, therefore, possible that the auxin in tomato tissue is more readily extracted with alcohol than with ether.

Another possible explanation of the better yield with alcohol may be the process of drying and recovering the extract. The procedure used in the present study appears to offer a superior method for the rapid drying and complete recovery of auxin extracts. This is of particular importance with alcohol, which is more difficult to evaporate than ether.

Tomato fruit tissue from field grown plants, which was dried by grinding with Na_2SO_4 , or in a drying oven, and subsequently extracted with ether or alcohol, gave yields very similar to those reported in table 1. This would indicate that there is little or no advantage in drying tomato tissues which are to be

TABLE 2. Variation in auxin content of tomato fruit due to season of harvesting. Extraction at 26°C. from 2.0 grams of tomato tissue cut into 1/8-inch cubes.

Solvent	Date of picking fruit	Part of fruit	Diameter of fruit in cm.	Auxin yield during extraction periods			Total auxin yield TDC/g.
				1st 18 hrs.	2nd 2 days	3rd 2 days	
Ethanol	7/12	Carpel wall	4.0	850	600	505	1955
Ethanol	8/4	Carpel wall	4.0	380	0	0	380
Ethanol	9/18	Carpel wall	4.0	0	0	0	0
Ethanol	8/4	Placenta and seeds	4.0	3700	500	...	4200
Ethanol	9/18	Placenta and seeds	4.0	850	330	...	1180
Ethanol	8/4	Carpel wall	2.0	400	300	0	700
Ethanol	9/18	Carpel wall	2.0	0	0	0	0
Ethanol	8/4	Placenta and seeds	2.0	2400	400	...	2800
Ethanol	9/18	Placenta and seeds	2.0	700	0	...	700

extracted with organic solvents unless the tissue is to be stored for future extraction.

Variation in auxin content of tomato fruit due to season of harvesting.—Although considerable care was taken to use similar samples of tomato fruit material week after week, it soon became evident that fruit harvested in the latter part of the season contained less auxin than similar fruit harvested earlier in the year. This observation is borne out by the data in table 2, when ethanol was used as an extracting solution. Table 3 shows a similar downward trend in auxin from tomato tissue which was dried and later extracted with an alkaline water solution at pH 10.0.

This seasonal variation must be borne in mind when an attempt is made to compare data from extractions made at different times during the year.

Alkaline hydrolysis extraction: effect of temperature and rapidity of drying tomato tissue on the yield of auxin.—The use of organic solvents did not seem to give immediate and complete extraction of auxin from tomato tissue, and a series of tests were therefore conducted using the alkaline hydrolysis method

reported by Avery, Berger, and Shalucha (1941). Several attempts had been made previously to use this type of water-extraction technique with fresh tomato fruit, but the auxin yields had been low and variable. Thimann and Skoog (1940) also reported poor results when fresh samples of *Lemna* leaves were extracted in boiling water at different pH levels.

The use of the alkaline hydrolysis method with dried tomato tissue (table 3) gave higher yields than were secured with organic solvents. The preliminary tests were conducted at pH 10.0, an alkalinity similar to that used by Avery *et al.* (1941) with corn endosperm.

The data in table 3 indicate that the faster the sample of tomato fruit was dried, the greater the yield of auxin obtained on subsequent extraction. This result was secured in all tests irrespective of the date upon which the fruit was harvested.

The drying of watery tomato fruit tissue involved several problems. Drying at 40°C. was done by cutting the sample into 1/8-inch cubes and then placing them in open petri dishes in a drying oven. The rate

TABLE 3. Alkaline hydrolysis extraction: auxin yields from tomato fruit in TDC/g. following drying for different lengths of time and at different temperatures. Extraction for 18 hours at 26°C. of 2.0 grams (fresh weight) of ground tissue in water at pH 10.0. All extracts adjusted to pH 5.8 for *Avena* test. Duration and temperature of drying.

Fruit size and date harvested	Fresh sample (undried)	20 hours at 40°C.; 15 min. at 110°C.		
		24 hours at 40°C.	50 minutes at 110°C.	
2.-3. cm. diameter, July 30.	3850	2180	3200	7680
As above, second test.	4150	2380	3460	8040
Average	4000	2280	3330	7860
Fruit size and date harvested		2 hours at 105°C.		30 minutes at 160-180°C.
2.0 cm. diameter, September 14.		300		922
As above, second test.		420		1050
As above, third test.		327		818
Average		349		930

TABLE 4. *Alkaline hydrolysis extraction: effect of pH of extracting solution on the yield (in TDC/g.) of auxin from dried tomato fruit. Samples consisted of 2.0 grams (fresh weight) of tomato fruit 2.0-3.0 cm. in diameter, dried for 80 minutes at 100°C., and ground to a powder. Extraction with 3.0 ml. of water for 18 hours at 26°C. at pH values indicated below. All extracts adjusted to pH 5.8 for Avena test. Fruit picked July 30.*

Test number	pH of tissue and solution during extraction period				
	4.1	4.6	6.1	9.2	11.8
1	1800	2700	2190	4000	6000
2	1980	2760	2880	4180	7080
3	1700	2600	2760	3800	6960
Average	1827	2683	2610	3990	6680

of drying was rather slow even when the samples were spread in a thin layer and stirred occasionally. Considerable difficulty was also experienced by the tissue sticking to the bottom of the dish. As the temperature of the drying oven was increased, the sticking became even more serious. The problem was solved by cutting the tomatoes into thin slices which were threaded on fine stiff wires and then suspended horizontally in the drying oven. This technique resulted in free circulation of air around the samples, extremely rapid drying, and practically no sticking of tissue to the wires.

Alkaline hydrolysis extraction: effect of pH of extracting solution on the yield of auxin from dried tomato fruit.—Several series of tests were conducted to determine what pH value of the extracting solution would give the highest yields of auxin. The results of this investigation are summarized in table 4. The pH values recorded in the table were secured by adding 1 N H₂SO₄ or 1 N NaOH to the water in which the ground tissue was suspended. During the extraction period the pH of the more alkaline solutions gradually dropped, due to the acid nature of the tomato tissue which is highly buffered at pH 4.6. The pH values recorded in table 4 were those at the end of the extraction period.

From the data in table 4 it may be seen that the smallest amount of auxin was extracted with the pH 4.1 solution. Significantly more auxin was removed at pH 4.6 and 6.1, followed by still further increases as the solution became alkaline. The maximum yield was reached at pH 11.8. The addition of an excess

of NaOH beyond pH 11.8 reduced the amount of auxin which was extractable. The solution at pH 11.8 which gave the highest yields consisted of two parts Clark's borate buffer pH 9.6 and one part 1 N NaOH. This solution was used at the rate of 3.0 ml. to 2.0 gm. (fresh weight) of ground, dried tissue.

Alkaline hydrolysis extraction: the effect of temperature and duration of extraction period on the yield of auxin from dried tomato fruit.—After the optimum conditions for drying the sample and the best pH values for extraction had been determined, the temperature and duration of the extraction period were studied. In the original discussion of the water extraction procedure, Avery *et al.* (1941) reported that heating at 100°C. for 15 minutes was the most effective treatment for corn endosperm. With dried tomato fruit tissue a lower temperature gave better results.

The data in table 5 show that higher yields were obtained at 26°C. than at 65° or 100°C. These results are similar to those secured when organic solvents were used with fresh tissue. The data also show that about 75 to 80 per cent of the auxin is liberated after only 5 to 10 minutes exposure to the solvent. Maximum yields were obtained by an 18-hour exposure to the alkaline water solvent at 26°C. Table 7 substantiates the above data for fruit picked on September 18, and in addition shows that a 40-hour exposure at 26°C. resulted in lower yields of auxin than did exposures for 1 or 18 hours at the same temperature. It will be noted in this table that the yields after 1 and 18 hours exposure are identi-

TABLE 5. *Alkaline hydrolysis extraction: the effect of temperature and duration of extraction period on the yield (in TDC/g.) of auxin from dried tomato fruit. Extraction with 3.0 ml. of water at pH 11.8 from 2.0 grams (fresh weight) of tomato fruit 2.0 to 3.0 cm. in diameter, dried for 80 minutes at 100°C., and ground to a powder. Solution of pH 11.8 secured by using two parts Clark's borate buffer pH 9.6, plus one part 1 N NaOH. All extracts adjusted to pH 5.8. Fruit picked July 30.*

Test number	Duration of extraction period and temperature of solvent				
	5-10 min. at 26°C.	45 min. at 26°C.	18 hours at 26°C.	20 min. at 65°C.	55 min. at 100°C.
1	4500	6000	6420	4400	3680
2	4800	5280	6720	3240	4280
3	5280	5700	6480	3660	3900
Average	4860	5660	6540	3767	3953

TABLE 6. Ether extraction of auxin from water extracts of tomato fruit tissue. All samples dried at 160°–180°C. for 30–40 minutes and ground to a powder. First extraction in each case was with 3.0 ml. water at pH 11.8 from 2.0 grams of tissue (fresh weight). Extract cleared of ground tissue by filtering through glass wool, followed by adjusting to pH 5.8 for *Avena* test. Ether extraction of water extract consisted of washing 1.0 ml. with four 4 ml. volumes of ether followed by drying and mixing with agar for *Avena* test. Fruit picked September 18.

Tomato variety and treatment	Auxin yield TDC/g.
Mingold, 2.3–2.6 cm. diameter, extracted 18 hours at 26°C.....	1230
Ether extract of above water extract.....	1725
Mingold, 4.2–4.4 cm. diameter, extracted 18 hours at 26°C.....	1475
Ether extract of above water extract.....	2750
Cherry, 1.2 cm. diameter, extracted 18 hours at 26°C.....	2750
Ether extract of above water extract.....	4800
Cherry, 2.0 cm. diameter, extracted 18 hours at 26°C.....	1250
Ether extract of above water extract.....	2600

cal. This was also true in a number of other tests, but usually there was a slight increase in yield when the extraction period was for 18 hours. By using this longer period the samples can be weighed out and the extraction started on the afternoon of the day before the *Avena* test is conducted. This gives more time for the final steps in the process and seems to make the whole procedure more satisfactory and easier to carry out.

Purification of auxin extracts.—In his original ether extraction method, Boysen Jensen (1936) felt that at least partial purification of the extract was necessary. Goodwin (1939) presented similar conclusions.

In the course of the investigation being reported here, several series of extractions were “purified” according to the general procedure of Boysen Jensen, who washed his samples with ether. Such tests were employed as a means of detecting the presence of inhibitors in the water or ethanol extracts.

When the ethanol was evaporated from ethanol extracts of fresh tomato-fruit tissue, there usually remained several milliliters of a water extract. On several occasions part of this water extract was tested for auxin content directly, and another portion was washed with ether, and then the ether extract was dried and tested along with the water extract remaining after the above washing. In all cases the original water extract and the ether extraction from the water gave similar yields of auxin. The water, left after the ether extraction, exhibited no auxin activity. This would indicate that no inhibitors were present in the ethanol extract or, if present, they were equally soluble in ether and ethanol.

The water extracts were washed or extracted with ether by adding 5.0 ml. of ether to 1.0 or 2.0 ml. of water extract in a test tube. The entire contents of the tube were then repeatedly drawn into a 10.0 ml. pipette and forced out rapidly to break the water extract into fine droplets in the ether. The material was finally drawn into the pipette and held while the water settled out. The water extract was allowed to

drain back into the test tube and the ether extract was evaporated to dryness. The water extract was washed with four changes of ether as outlined above.

In another test of an ethanol extract, sufficient indoleacetic acid was added to one sample to give an additional calculated 1000 TDC per gram of original extract. The *Avena* tests in this case indicated 300 TDC per gram for the original extract and 1400 TDC per gram for the extract plus indoleacetic acid. These results indicate that either no inhibitors are present or that they have no additional inhibiting effect on the indoleacetic acid.

Similar tests were conducted with water extracts of dried tomato tissue. One-milliliter portions of the water extracts were washed with ether, as previously discussed for the ethanol extract. As indicated in tables 6 and 7, such ether extracts gave appreciably higher auxin yields than the original extract. When the ether was used with an alkaline water extract, no auxin was dissolved by the ether. When the water extract was acidified, the auxin yield of the dried ether extraction was from 25 to 100 per cent higher than the original water extract.

The superiority of the ether extracts over the original water extract might suggest the presence of an inhibitor, although it is not conclusive evidence on the matter. The water extracts from the dried tissue were dark brown in color and, no doubt, contained many impurities. The solutions were cleared of ground plant tissue by centrifuging or by filtering by suction through a pad of grass wool in a small funnel, but this did not remove dissolved impurities in the extract. Agar blocks containing these extracts were cloudy and discolored. Agar blocks containing the dried residue of the ether extractions were clear. The diffusion of auxin through the cloudy block with the apparently large quantity of impurities may have been slower than in the clearer blocks obtained following the ether extraction procedure.

Enzymatic liberation of auxin from dried tomato fruit tissue.—The use of proteolytic enzymes in the water extraction of auxin from plant tissue was re-

TABLE 7. *Enzymatic liberation of auxin from dried tomato fruit tissue. Samples consisted of 2.0 grams of tissue (fresh weight) dried at 160°–180°C. for 30–40 minutes. Solvent in each case had a total volume of 3.0 ml. adjusted to the pH indicated with 1 N NaOH. Enzymes consisted of 1.0 mg. in above volume of solvent. All fruit was 2.0 to 3.0 cm. in diameter.*

Treatment	Auxin yield TDC/g.
Fruit picked July 30	
Water at pH 11.8; 24 hours at 40°C.....	3300
Chymotrypsin at pH 8.0; 24 hours at 40°C.....	7000
Papain at pH 4.6; 24 hours at 40°.....	3400
Trypsin at pH 8.0; 24 hours at 40°C.....	1500
Water at pH 11.8; 12 hours at 26°C.....	6700
Chymotrypsin at pH 8.0; 12 hours at 26°C.....	4500
Papain at pH 4.6; 12 hours at 26°C.....	2400
Water at pH 11.8; 15 hours at 26°C.....	7000
Chymotrypsin at pH 8.0; 15 hours at 26°C.....	6400
Papain at pH 4.6; 15 hours at 26°C.....	2800
Fruit picked September 18	
Water at pH 11.8; 40 hours at 26°C.....	1800
Ether extract of above water extract.....	2200
Water at pH 11.8; 18 hours at 26°C.....	3000
Ether extract of above water extract.....	3400
Water at pH 11.8; 1 hour at 26°C.....	3000
Ether extract of above water extract.....	3500
Chymotrypsin at pH 8.0; 40 hours at 26°C.....	2700
Chymotrypsin at pH 8.0; 18 hours at 26°C.....	2400

ported by Skoog and Thimann (1940). Several series of tests were conducted in the present investigation to study their effectiveness when used with dried tomato-fruit tissue. The extraction procedure was similar to that used in the alkaline hydrolysis tests. Two grams (fresh weight) of dried tissue were used in each test, plus 3.0 ml. of solvent. The solvent consisted of water plus 1.0 mg. of enzyme adjusted to the pH value at which the enzyme was known to be most active. This provided optimum conditions for each different enzyme as follows: chymotrypsin, pH 8.0; trypsin, pH 8.0; and papain, pH 4.6. At the end of the extraction periods the solutions were cleared of ground tissue by centrifuging or filtering through glass wool. The pH was then adjusted to 5.8 for the *Avena* test.

The results of the enzyme extraction experiments are recorded in table 7. In these tests trypsin and papain did not give as high yields of auxin under any condition of temperature or exposure as did an alkaline water solution at pH 11.8. At 40°C. the chymotrypsin at pH 8.0 and water at pH 11.8 gave approximately equal yields from fruit picked on July 30. Chymotrypsin solution was slightly less effective than the alkaline water solution for extracting auxin from the fruit harvested on September 18.

The above results seem to justify the conclusion that trypsin and papain will not increase the yield of auxin from dried tomato tissue. The extraction of dried tomato-fruit tissue with chymotrypsin solu-

tion at pH 8.0 gave equal or slightly lower yields of auxin than alkaline water at pH 11.8.

Auxin extraction from different sizes and varieties of tomato fruit.—The tests reported in this paper indicate that auxin may be extracted from dried tomato-fruit tissue by the use of a modification of Avery's alkaline hydrolysis procedure. In order to make a preliminary application of these findings to a problem of horticultural interest, a number of extractions were made to determine the variation in hormone content between different varieties and sizes of tomato fruit.

The standard extraction procedure used in these tests was as follows. As soon after picking as possible the fruits were cut into thin slices, weighed, and placed on wires in the drying oven at 160° to 180°C. When drying was completed after 30 to 40 minutes, the slices were placed in weighing dishes, cooled in a desiccator, and weighed for dry-weight determinations. The dry samples were then ground to a fine powder and returned to the desiccator until needed for extraction.

On the day before the *Avena* test was to be carried out a 2.0 g. sample (fresh weight) of tissue to be extracted was placed in a test tube and 3.0 ml. of a mixture of 2 parts Clark's borate buffer pH 9.6 and 1 part 1 N NaOH was added. After carefully mixing the ground tissue and extracting solution, the samples were placed at a constant temperature of 26°C. for 18 hours. In the writer's laboratory the extractions were started at 2:00 P.M. At 8:00 A.M.

TABLE 8. *Auxin extraction (in TDC/g.) from different sizes and varieties of field grown tomato fruit. Samples dried at 160°-180°C. for 30 to 40 minutes and ground to powder. Two grams of tissue (fresh weight) extracted with 3.0 ml. water at pH 11.8 for 18 hours at 26°C. Water extract, cleared of ground tissue by filtering through glass wool, adjusted to pH 5.8 and 1.0 ml. extracted with four 4 ml. volumes of ether. Each figure represents the average of duplicate racks of 12 *Avena* plants tested on at least 3 different days from a new extraction on each day. Fruit picked September 18.*

Fruit diameter in cm.	Stage of maturity	Part of fruit extracted		
		Entire	Placenta and seeds	Carpel wall
Stone (red) variety of tomato				
1.5	green	800	1020	300
3.0	green	935	2350	260
5.0-5.5	green	1735	2197	340
5.0-5.5	half ripe	1260	260
Mingold (yellow) variety of tomato				
2.3-2.6	green	1725
4.2-4.4	green	2750
5.0-5.5	green	660
Sugar (Cherry) variety of tomato				
1.2	green	4800
1.8-2.0	green	2600
2.5	half ripe	1100

the following morning the solutions were cleared of the ground material by filtering through glass wool and the pH was adjusted to pH 5.8. One milliliter of the extract was then washed four times with 4.0-ml. volumes of ether. The ether extract thus secured was dried at 50°C., and sufficient 1.5 per cent agar was added to give the proper dilution for the *Avena* test. Three different dilutions of each sample were made to be sure the results were within the proportionality range.

Table 8 presents the summarized results of a number of extraction tests from different sizes and varieties of tomato fruit. The fruit was all picked on the same day and dried and extracted under identical experimental conditions. The results of these tests furnish further evidence that the modified alkali-hydrolysis method presented here offers the best procedure for auxin extraction from tomato fruit tissue which has been published up to the present time. It is not suggested that the method extracts the total amount of auxin and precursor which is present in the tomato, but the yields are definitely higher than any previous reported.

This method has several advantages in addition to higher auxin yields. The tissue from which the extraction is made is dried and therefore relatively stable. The extraction may be made from the dried samples over a period of several weeks which makes it possible for one person to test several types of tissue at different stages of development which would be difficult or impossible if all extractions had to be made from fresh material.

The data in table 8 show that differences in the auxin content of similar tissues were determined by alkali-hydrolysis extraction of dried samples and

indicate that the highest auxin content per gram of fresh tissues of the Stone variety of tomato is when the fruit is from 3.0 to 5.0 cm. in diameter. This period of highest auxin content corresponds very closely to the period of most rapid growth as reported by Judkins (1940). Similar trends in auxin content are also exhibited in Mingold and Sugar varieties. The data for the Sugar variety in table 8 might not seem to bear out this statement, but this is a small-fruited tomato which is slowing down markedly in its growth rate by the time it is 2.0 cm. in diameter.

SUMMARY

A method is described for the rapid drying (in test tubes) and complete recovery of organic solvent extracts of auxin.

The use of ethanol as a solvent in the extraction of auxin from fresh tomato tissue gave higher yields than ether, acetone, methanol, dioxane, or ethyl acetate.

A number of successive extractions of the same sample of tissue are necessary to secure all of the extractable auxin with ethanol, ether, or acetone. Methanol, dioxane, and ethyl acetate gave very low or no yield of auxin at the first extraction and no further yields on subsequent extractions.

The extraction of auxin from dried tomato tissue by the use of water at pH 11.8 for 18 hours at 26°C. was found to give higher yields than any other method used by the author, or previously reported in the literature. When the above extract was adjusted to pH 5.8 and extracted with ether, the yield of auxin was increased still further. The above method is presented in detail and used in a brief

study of the auxin content of different sizes and varieties of tomatoes.

The more rapidly the tomato tissue was dried in preparation for the above extraction technique, the higher was the subsequent yield of auxin. A method of drying tomato tissue on wires at a temperature of 160°–180°C. was developed and gave excellent results in the above mentioned alkali-hydrolysis method of auxin extraction.

The use of chymotrypsin in the extraction of auxin from dried tomato tissue gave yields equal to, or slightly lower than, water at pH 11.8. Papain was approximately one-half and trypsin one-fourth as effective as chymotrypsin.

The hormone content per gram of fresh tissue of

tomato fruit was found to be highest during the early part of the season when the plants were growing vigorously, and lowest toward the end of the season when the plant growth was slower.

A brief study of different sizes and varieties of tomato fruit shows that the alkali hydrolysis method proposed in this report gave a quantitative index of the variation in hormone content. The highest yields of auxin were obtained during the period of most rapid increase in fruit size. This condition is the same for Stone, Mingold, and Sugar varieties of tomato.

DEPARTMENT OF HORTICULTURE,
OHIO AGRICULTURAL EXPERIMENT STATION,
WOOSTER, OHIO

LITERATURE CITED

- AVERY, G. S., JR., J. BERGER, AND B. SHALUCHA. 1941. The total extraction of free auxin and auxin precursor from plant tissue. *Amer. Jour. Bot.* 28: 596–607.
- , ———, AND R. O. WHITE. 1944. Rapid extraction of free auxin and auxin precursor from green plant tissue. *Amer. Jour. Bot.* 31: 8s.
- , H. B. CREIGHTON, AND B. SHALUCHA. 1940. Extraction methods in relation to hormone content of maize endosperms. *Amer. Jour. Bot.* 27: 289–300.
- , ———, AND ———. 1941. Expressions of hormone yields in relation to different *Avena* test methods. *Amer. Jour. Bot.* 28: 498–506.
- BOYSEN JENSEN, P. 1936. Über eine Micromethode zur quantitativen Bestimmung der Wuchsstoffe der A-Gruppe. *Planta* 26: 584–594.
- GOODWIN, R. H. 1939. Evidence for the presence in certain ether extracts of substances partially masking the activity of auxin. *Amer. Jour. Bot.* 26: 130–134.
- GUSTAFSON, F. G. 1939. Auxin distribution in fruits and its significance in fruit development. *Amer. Jour. Bot.* 26: 189–194.
- JUDKINS, W. P. 1940. Time involved in pollen tube extension through style and rate of fruit growth in tomato (*Lycopersicum esculentum* Mill.). *Proc. Amer. Soc. Hort. Sci.* 37: 891–894.
- LAIACH, F., AND F. MEYER. 1935. Über die Schwankungen des Auxingehaltes bei *Zea Mays* und *Helianthus annuus* im Verlauf der Ontogenese. *Senckenbergiana* 17: 73–86.
- LINK, G. K. K., V. EGGERS, AND J. E. MOULTON. 1941. Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs: Its extraction with dry ether. *Bot. Gaz.* 102: 590–601.
- LINSER, HANS. 1939. Zur Methodik der Wuchsstoffbestimmung. II. Die Extraktion von Pflanzenmaterial. *Planta* 29: 392–408.
- VAN OVERBEEK, J. 1938. A simplified method for auxin extraction. *Proc. Nat. Acad. Sci. (U.S.A.)* 24: 42–46.
- SKOOG, F. 1937. A deseeded *Avena* test method for small amounts of auxin precursors. *Jour. Gen. Physiol.* 20: 311–334.
- , AND K. V. THIMANN. 1940. Enzymatic liberation of auxin from plant tissue. *Science* 92: 64.
- THIMANN, K. V. 1934. Studies on the growth hormones of plants. VI. The distribution of the growth substances in plant tissue. *Jour. Gen. Physiol.* 18: 23–34.
- , AND F. SKOOG. 1940. The extraction of auxin from plant tissues. *Amer. Jour. Bot.* 27: 951–960.
- WENT, F. W. 1928. Wuchsstoff und Wachstum. *Rec. Trav. Bot. Néerland.* 25: 1–116.

THE ANATOMY OF LEAF ABSCISSION AND EXPERIMENTAL DEFOLIATION IN GUAYULE¹

Fredrick T. Addicott

THE LEAVES of guayule (*Parthenium argentatum* A. Gray) contain relatively low amounts of rubber. They also contain appreciable amounts of materials that lower the quality of milled rubber when the leaves are left on the shrub during milling. Hence defoliation is a desirable step in the improvement of the milling process. Several methods of defoliation have been under consideration: one involves flash-drying followed by tumbling; another consists of immersing the plants in boiling water for a few minutes, after which the leaves fall off with a slight agitation; a third possibility was developed from experiments with the retting of intact shrubs. The anatomical aspects of these processes were studied. Since very little was known of the anatomy of leaf abscission in guayule, it was considered advisable first to investigate this process in field plants. The results of both phases of the investigation are reported in this paper.

There is a considerable body of knowledge concerning the physiology and anatomy of leaf abscission. Pfeiffer (1928) in his review of the subject gives a list of citations covering 18 pages. However, there appears to have been very little work done on plants such as guayule whose abscission layer does not function actively in leaf fall. Certain of the anatomical changes that take place at the petiole base of *Rhus typhina* (Lee, 1911) and *Mercurialis annua* (Yampolsky, 1934) as well as in the perigone and style of *Narcissus pseudonarcissus* (Namikawa, 1926) are similar to those at the petiole base in guayule. Artschwager (1943), in his anatomical study of guayule, did not consider the abscission of leaves; however, Lloyd (1911) refers briefly to the environmental conditions affecting leaf-fall as well as to the absciss layer of leaves. The absence of details on the anatomy of leaf-fall in guayule, the general lack of information on similar plants, and the need for knowledge fundamental to the development of methods of defoliation justifies a careful examination of the problem in this plant.

MATERIALS AND METHODS.—Leaf bases of field plants were obtained from the Hansen and Lee Field of the Salinas, California, plantings of the Emergency Rubber Project. The plants were two years old; the field was irrigated. Samples from flash-drying and retting experiments were obtained through the courtesy of the Guayule Rubber Extraction Research Unit. Samples for the study of the action of hot water in defoliation were obtained from small-scale experiments carried on in the laboratory with material from field plants. Tissue

was fixed in formalin-propionio-ethanol (Johansen, 1940), sectioned in paraffin, and stained by the safranin-hematoxylin method of Esau (1944).

DEFINITION OF TERMS.—*Abscission*—a natural process by means of which an organ is shed.

Defoliation—this term is used variously in the literature, but in this paper it is restricted to indicate the artificial removal of leaves; that is, the removal of leaves by agents under the control of man.

Abscission layer—a layer which develops at the base of an organ and which assists in either the separation of the organ or the protection of underlying tissues, or both.

Abscission zone—a region of weakness at the base of the petiole of guayule. When a leaf separates from the stem the break occurs within this zone. The cells of the abscission layer form only a part of the abscission zone.

LEAF ABSCISSION IN THE FIELD.—Lloyd (1911) briefly described the cycle through which the leaves of guayule pass. He considered the shrub semi-deciduous because, although the leaves are never all shed at one time, there are periods of greater and lesser fall. The principal leaf-fall occurs during the autumn months. By February a relatively static situation has been reached with clusters of small winter leaves remaining at the tips of the branches. These terminal leaves are shed soon after the resumption of shoot growth in the spring so that under field conditions in Salinas all of the preceding season's leaves have fallen by the end of May. The expression "leaf fall" as used here does not imply, however, that the leaves drop from the plant readily. Under many circumstances they may die and remain attached to the stem indefinitely. Wind, other external forces, and the enlargement of the stem in growth would appear to be the principal agencies by which dead leaves are ultimately removed from the plants.

Lee (1911) credits von Mohl with pointing out that there are two sets of phenomena connected with leaf-fall: *separation* and *protection*. In guayule there is no physiological mechanism of separation but there are, on the other hand, distinct anatomical mechanisms for protection. Before the leaf falls the following structural changes occur: the resin canals which join the petiole and the cortex become plugged with a mass of parenchyma (fig. 4-6); an abscission layer differentiates across the parenchymatous tissues (fig. 5-7); and the cells of this layer adjacent to the leaf trace begin the obliteration of the conductive tissues (fig. 6, 8). No indication of digestion of middle lamellae or of cells to bring about the separation of the leaf was observed. The details of the anatomical changes during the de-

¹ Received for publication January 20, 1945.

The writer wishes to express his appreciation to Miss Margaret A. Ewing and Mrs. Jeanne B. Pankhurst for their wholehearted assistance in this work.

velopment of the protective tissues at the site of the future leaf-scar are described below.

In longitudinal sections through the base of the petiole and adjacent stem the most conspicuous structures are the leaf trace, resin canals and abscission layer (fig. 2-7). The leaf bundles in the petiole are accompanied by abaxial and adaxial groups of fibers as are the leaf traces in the stem. However, these fibers are lacking in the region of union between the petiole and stem. Lee's (1911) figures show many similar cases in other species. In the petiole there are usually three but sometimes five bundles. Each is paralleled by two resin canals. One canal is located abaxially and the other adaxially to each bundle about midway between the bundle and the epidermis. Those on the adaxial side come to a finely tapered end just short of the base of the petiole and do not connect with the canals in the cortex (fig. 3). The resin canals accompanying the veins on their abaxial sides continue into the cortex (fig. 3). However, these canals become plugged with a mass of parenchyma by the time the leaf is fully matured (fig. 4-6). Thus the resin canal systems in the mature leaf have no connection with those in the cortex.

The resin canal plug develops relatively early in the ontogeny of the leaf. It has not been possible to determine from external criteria the stage in which it appears but it is present soon after the leaf may be considered fully expanded or mature. Its development is apparently rapid. Of more than 100 leaf bases collected and sectioned with the hope of finding intermediate stages in the development of resin canal plugs, only a few early stages were found (fig. 4). For the most part the resin canals were either open (fig. 3) or had a well developed plug (fig. 5, 6). Limited observations suggest that the resin canal plug develops by the enlargement and division of cells lining the canal. At first the plug is entirely parenchymatous, but later the central portion becomes suberized and forms a part of the abscission layer (fig. 5, 6). The plug may be similar in nature to the "pseudo-tyloses" described by Lloyd (1911) which consist of groups of cells varying from small protuberances made up of one or two cells to masses blocking the canals. Lee (1911) presents a figure of *Rhus typhina* which shows a resin canal plug similar to that in guayule, but he does not discuss this structure in his text.

The function of the resin canal plug would appear to be largely protective, in both preventing the loss of resin and the possible entry of pathogenic organisms after the leaf has fallen. The presence of the plug in the resin canal adds to Lloyd's (1911) observation that there are many independent resin canal systems in guayule. For the latter part of the life of the leaf its resin canals have no connection with any of the canals in the rest of the plant. A somewhat similar situation exists in the genus *Picea* where Plavšić (1934) has found no union between the resin canals of the leaf and those in the cortex.

The beginnings of the abscission layer were de-

tected at an early stage in its development (fig. 5). Microchemical tests with Sudan IV and oil blue NA indicated that suberin was present in this layer as early as in the adjacent periderm. As in the olive (Hewitt, 1938) and probably many other species, the abscission layer develops simultaneously with the periderm in the surrounding cortex. The suberized cells of the abscission layer are similar in appearance to those of the phellem developing from the phellogen. However, the abscission

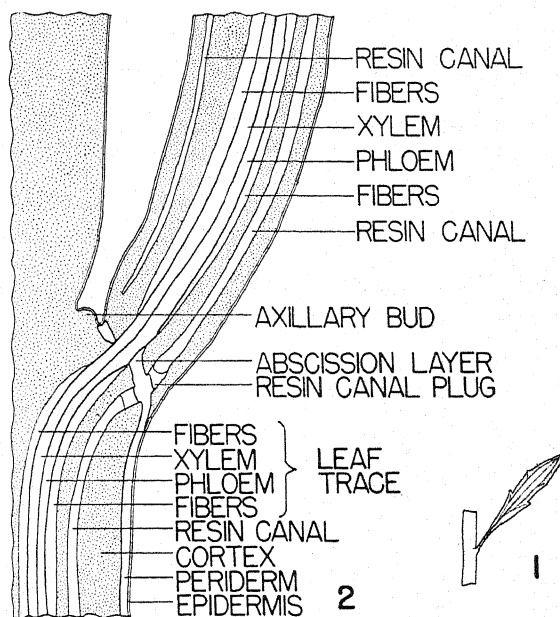


Fig. 1-2.—Fig. 1. Sketch of leaf and adjacent stem. $\frac{1}{2}$ natural size.—Fig. 2. Diagrammatic median longitudinal section through the base of the petiole and adjacent stem. Magnification about 30 \times .

layer develops by the differentiation of already existing parenchyma cells (fig. 5-7). Therefore the shape of its cells is less regular than that of periderm cells. A phellogen was not detectable on the inner side of the abscission layer until after the leaf had died. No instance has been observed in which the cells of the abscission layer were oriented so that they might present a continuously smooth surface for a future leaf scar. Adjacent to the phloem the abscission layer increases in width and develops the shape of a lens (fig. 6). By the time the leaf is yellow the lens is quite conspicuous (fig. 8). The pressure of enlarging cells leads to compression of the phloem. Sections have been observed in which the width of the phloem has been reduced at least thirty per cent. It is possible that this phenomenon may be in part responsible for the death of the leaf.

Examination of the scars left by recently fallen leaves disclosed further development of the abscission layer in the leaf trace. Xylem parenchyma and adjacent cells in the cortex enlarge and deposit suberin in their walls. This occurs a short distance

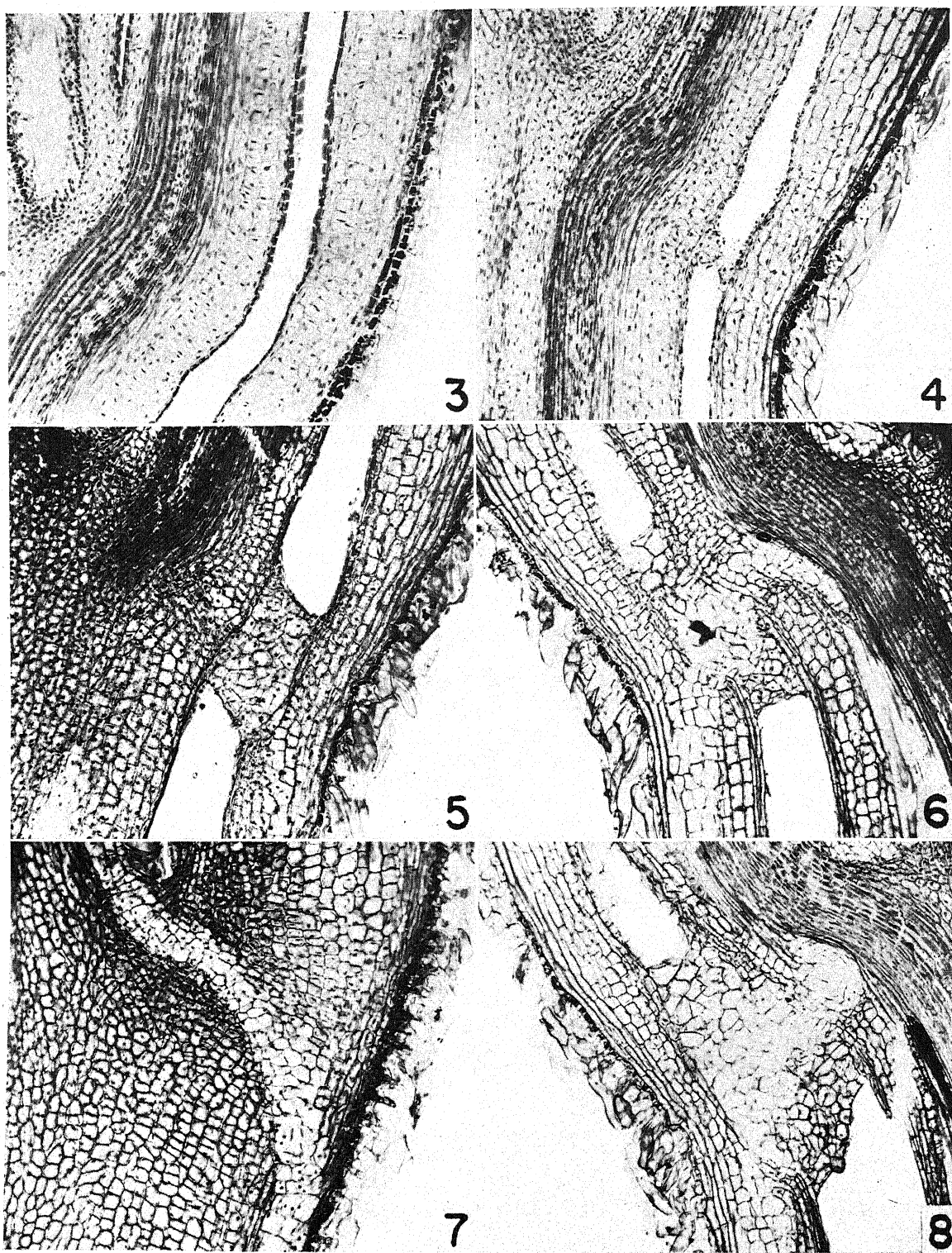


Fig. 3-8. Photomicrographs of longitudinal sections of the leaf base. $\times 85$.—Fig. 3. Section from a young leaf showing the unplugged abaxial resin canal and the termination of the adaxial resin canal.—Fig. 4. Resin canal plug in early stage.—Fig. 5. Resin canal plug with the abscission layer developing in the plug and adjacent tissues.—Fig. 6. Later stage showing a well developed abscission layer.—Fig. 7. A non-median section showing the continuity of the abscission layer with the adjacent periderm.—Fig. 8. Late stage showing expansion of the resin canal plug and compression of phloem in leaf trace.

inward from the surface of the scar (fig. 9, 10). Sealing of the xylem appears to be accomplished through the resorption of vessels by the adjacent parenchyma cells as they enlarge (fig. 10). At the surface of a scar there is a region of dead tissue and more or less intact xylem. Collapse and resorption of the vessels increases inwardly to a point where they are barely detectable. Below this point vessels can be found again (fig. 9, 10). No crushing or tearing of vessels was observed in the abscission zone. Rather the vessels appeared to have been softened and eroded by the developing abscission layer, and the wall materials of the vessels incorporated in the walls of the layer. As the vessel walls are resorbed the adjacent cells enlarge and fill the space formerly occupied by the vessels. The position of the former vessels can usually be detected by local thickenings of the suberized walls of the former parenchyma. Such thickenings have often been seen to be continuous with more or less intact vessels adjacent to the region of resorption. After the leaf is dead a phellogen appears along the inner margin of the abscission layer. This leads to the development of a periderm to the inside of the abscission layer which is continuous with the periderm in other parts of the stem. At the site of the scar there are therefore the protective tissues of a suberized abscission layer and a periderm which appears immediately beneath it.

Only two indications of resorption of vessels have been found in the literature consulted. Namikawa (1926) recorded the observation of crushed tracheids in *Narcissus pseudo-narcissus*. Yampolsky (1934) shows a figure of *Mercurialis annua* which seems to indicate that the xylem has been resorbed, but he does not discuss this aspect of the figure. It seems possible that the phenomenon of vessel resorption may be much more widespread than is now apparent.

Examination of leaf scars always discloses a surface of broken cells (fig. 9). The break usually passes through the abscission zone at the base of the petiole just external to the abscission layer. Often a few cells of the abscission layer are carried away with the falling leaf, but the continuity of the layer is seldom broken. A few instances have been observed in which the break occurred in the petiole several millimeters from the stem. Usually the break occurs in a well defined zone at the base of the leaf. Here the petiole is flattened and clasping, and the strengthening fibers associated with the leaf trace in the stem and leaf bundle in the petiole are lacking (Eames and MacDaniels, 1925). Thus the break does not involve any well defined layer, but may pass through any part of a region of weakness. This region is called the abscission zone.

Discussion.—Whether leaf fall in guayule should be considered a process of abscission, in the sense usually understood may be questioned. Observations of field material indicate that leaf fall occurs only after the death of the leaf and after the tissues

have become more or less brittle. The abscission layer is not oriented so as to present a smooth surface for the leaf scar, and usually is not involved in the break that occurs when the leaf falls. Furthermore, no evidence was found of physiological activity at the abscission layer such as the digestion of middle lamellae, cell walls or layers of cells.

Namikawa (1926) has suggested the term exfoliation to cover cases of this type. His definitions follow: "The term exfoliation is to be understood as implying the falling of a certain organ, being preceded by the death of it, and accomplished by the mechanical rupture of dead tissue. . . . Abscission means, on the other hand, the amputation of an organ by means of isolation of living cells in a special separation-layer usually predetermined in a certain part of the organ." Namikawa's distinctions between exfoliation and abscission may be more artificial, at least when applied to leaves, than a cursory examination would indicate. The process of abscission in guayule does fit his definition of exfoliation. On the other hand, the anatomical structures involved with leaf fall closely resemble the layers concerned with abscission in many other plants (Eames and MacDaniels, 1925; Hewitt, 1938). Guayule, therefore, does not fit completely into either of Namikawa's categories. Thus in considering the phenomenon of leaf fall it is suggested that we retain the use of the word abscission in its original and broad sense to apply to all cases. Within this single category there would be many variations, among which guayule is only one.

Of the two functions of abscission layers, separation and protection, only one is shown by the abscission layer of guayule. The function of this layer appears to be entirely protective. It is well suberized before the leaf falls, and completely covers the entire area of the scar, including the resin canals and leaf traces. No evidence of its physiological activity in separation was observed. However, the development of the abscission layer may have indirect physiological effects especially through its constriction of the phloem.

Defoliation by means of flash-drying.—Leaves can be removed from harvested guayule by exposing the shrub to heated air for a few minutes and then tumbling it in a revolving drum. This removes a high percentage of the leaves, but also a number of the smaller twigs. An occasional tuft of leaves in which the blades are closely appressed is not removed by this method. Examination of material in the process of treatment indicated that the flash-drying method accelerates the natural process by rapidly rendering the leaves and petioles brittle.

Microscopic examination of flash-dried material disclosed that the tissues had been killed by the dehydration. Defoliation was accomplished by a break through the abscission zone as under field conditions. However, the break was more clean-cut (fig. 11, 12). Microchemical examination of twigs lost in tumbling, using a differential stain for rubber (Addicott, 1944), showed appreciable amounts

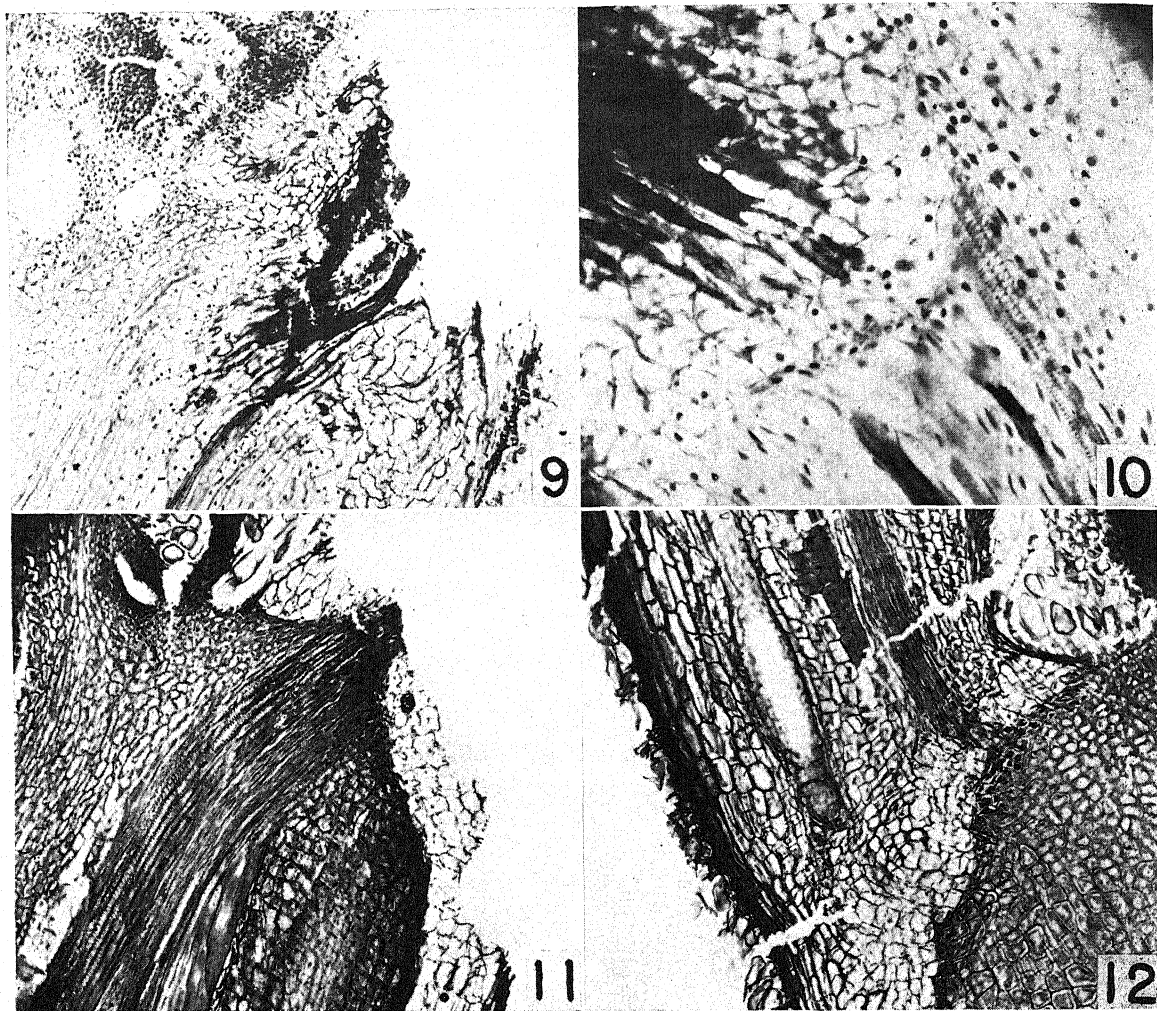


Fig. 9-12. Longitudinal sections of leaf bases and leaf scars. $\times 85$, except figure 10 which is $\times 170$.—Fig. 9-10. Sections of natural leaf scars. Note xylem resorption in the abscission layer. Beneath this periderm has appeared.—Fig. 11. Section of a leaf scar produced in a flash-drying experiment. Note that the break has passed *across* cells of the abscission layer.—Fig. 12. Section of leaf which had been flash-dried, but which had not been removed by tumbling. Note incomplete breaks which pass *across* cells.

of rubber. Economic considerations as well as the disadvantages mentioned have recently led to the cessation of experiments with the flash-drying method of defoliation.

Defoliation by means of flash-boiling.—If guayule shrubs are immersed in boiling water for several minutes most of the leaves fall off readily. A few vigorous shakes are sufficient to defoliate the plants entirely. The amount of energy required to remove a leaf after the boiling water treatment is considerably less than after flash-drying.

Microscopic observations showed that under this treatment the break also passed through the abscission zone. However, the break almost always passed between cells, rarely across them (fig. 13). Apparently the hot water softens the middle lamella to such an extent that the cells part readily. Microchemical tests to check this point by the use of dyes

usually considered to be more or less specific for pectic substances were inconclusive. The cell walls of the parenchyma were so impregnated with pectins that it was impossible in most cases to detect the middle lamella. However, the fact that, when separation occurred, it came between cells and left intact walls on either side, strongly suggests that it was the middle lamella which gave way. The increased effectiveness of the flash-boiling method has led to its favorable consideration as a method of defoliation.

Defoliation by means of retting.—In connection with certain experiments of the Guayule Rubber Extraction Research Unit it was observed by Dr. E. P. Jones that immersion in water for several days at room temperature, or confinement under conditions of high humidity for about two weeks led to changes at the leaf base which permitted the

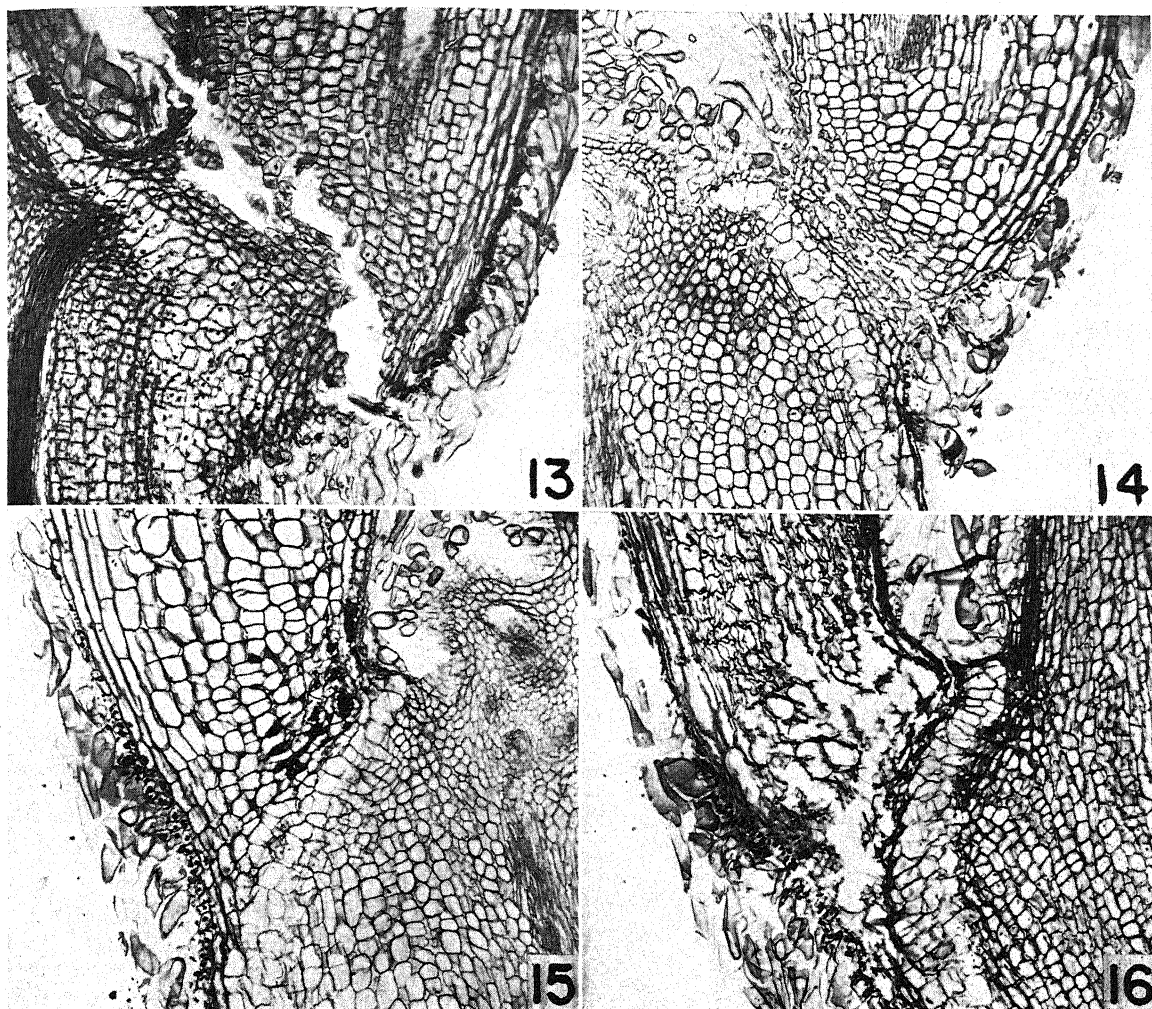


Fig. 13-16.—Longitudinal sections of leaf bases from experiments on defoliation. $\times 85$.—Fig. 13. Section of leaf base subjected to hot water treatment. Break passes *between* cells.—Fig. 14. Section from immersed shrub. Note only partial collapse of cells at base of petiole, and their separation.—Fig. 15. Section of shrub from humid conditions. Note pockets of microorganisms.—Fig. 16. Section of shrub from humid conditions. Note severe collapse of cells with disintegration adjacent to the abscission layer.

ready defoliation of the shrubs. In each case an abundant microbic flora developed. The odor of the immersed shrub indicated that large numbers of putrefactive bacteria were present. The plants confined under humid conditions showed a profuse development of molds especially on the leaves and smaller branches.

Microscopic examination of samples from both treatments disclosed in each case the widespread occurrence of microorganisms within the tissues at the base of the petiole (fig. 15, 16). Sections from shrubs under humid conditions showed a considerable breakdown of the cells. In many cases the softer tissues, particularly just outside the abscission layer, were disintegrated by the end of two weeks (fig. 16). Collapse of cells was rarely evident in the immersed shrubs. After this treatment the cells remained more or less intact,

but separated readily (fig. 14). It is interesting to note that in each treatment the abscission layer was hardly affected. The collapse or separation of cells occurred almost entirely outside the layer.

Whether moisture or the presence of microorganisms was the more effective in defoliation could not be determined from observations. It is possible that both may be important. In view of the rapid action of boiling water in defoliation it seems logical to assume that exposure to water at room temperatures might have a similar effect if the treatment were sufficiently prolonged. The separation of cells observed (fig. 14) resembles the condition found after flash-boiling (fig. 13). Although the microorganisms present were not identified it seems likely that the marked injury and destruction of cells observed can be accounted for only by the activity of molds or bacteria. Both

moisture and microorganisms, therefore, may function in defoliation by retting. The possibility remains of course that other factors may also be involved. The time and storage facilities required to defoliate guayule by retting suggest that it would not be economical to employ this method.

It may be of interest to note that whereas in guayule no separation of cells occurs during abscission in the field, it does occur under the artificial conditions of two of the defoliation methods. Under field conditions the separation of the leaf appears to be entirely mechanical with cells being broken and the abscission layer having no direct part. Under the conditions of flash-boiling and of retting by immersion a separation of cells is achieved (fig. 13, 14). This is analogous to the situation in the classical type of abscission in which the middle lamellae of cells of the abscission layer are digested by physiological action. However, the parallel is not close; in guayule the cells which separate under the above treatments are not a part of the abscission layer but lie in the petiole base just outside the abscission layer.

SUMMARY

Leaf fall from guayule under field conditions is considered to be a modified type of abscission. The abscission layer is not directly involved in the separation of the leaf from the stem. Separation is mechanical and occurs after the leaf dies

by a break which passes through the weak abscission zone at the base of the leaf. There is no indication that physiological action, such as the digestion of middle lamellae, or cells, is involved in the freeing of the leaf.

Protection for the stem is provided by the abscission layer and the periderm which develops beneath it. The abscission layer forms at the leaf base in cortical parenchyma, resin canal plugs, and within the leaf trace. It is continuous with the periderm of adjacent regions of the stem and is well suberized before the leaf falls. Cells of the abscission layer developing within the leaf trace resorb and replace the vessels for a short distance. A phellogen appears immediately beneath the abscission layer about the time of the death of the leaf.

Defoliation in the flash-drying process is accomplished by a break passing across the cells of the abscission zone after the tissues have been made brittle by dehydration.

Defoliation by the flash-boil method apparently results from the softening of the middle lamellae. When leaves are removed after this treatment the break passes between cells.

SPECIAL GUAYULE RESEARCH PROJECT,
UNIVERSITY OF CALIFORNIA,
SANTA BARBARA COLLEGE,
SANTA BARBARA, CALIFORNIA

LITERATURE CITED

- ADDICOTT, FREDRICK T. 1944. A differential stain for rubber in guayule. *Stain Tech.* 19: 99-102.
- ARTSCHWAGER, ERNST. 1943. Contribution to the morphology and anatomy of guayule (*Parthenium argentatum*). U. S. Dept. Agric. Tech. Bull. No. 842. 1-33.
- EAMES, ARTHUR J., AND LAURENCE H. MACDANIELS. 1925. An introduction to plant anatomy. McGraw-Hill, New York.
- ESAU, K. 1944. Anatomical and cytological studies on beet mosaic. *Jour. Agric. Res.* 69: 95-117.
- HEWITT, W. B. 1938. Leaf-scar infection in relation to the olive knot disease. *Hilgardia* 12: 39-71.
- JOHANSEN, D. A. 1940. Plant microtechnique. McGraw-Hill, New York.
- LEE, E. 1911. The morphology of leaf fall. *Ann. Bot.* 25: 51-107.
- LLOYD, F. E. 1911. Guayule, a rubber plant of the Chihuahuan desert. *Carnegie Inst. Washington Pub.* No. 139: 1-213.
- NAMIKAWA, ISAWO. 1926. Contributions to the knowledge of abscission and exfoliation in floral organs. *Jour. Coll. Agric. Hokkaido Imp. Univ.* 17: 63-131.
- PFEIFFER, HANS. 1928. Die pflanzlichen Trennungsgebe. *Handbuch der Pflanzen-Anatomie.* Abt. 1, Teil 2, Bd. 5, Leif 22. Gebrüder Bornträger, Berlin.
- PLAVŠIĆ, SVETISLAV. 1934. Über die Harzkänale in Blattkissen der Gattung *Picea*. *Beih. Bot. Centralbl. Abt.* A. 52: 290-297.
- YAMPOLSKY, CECIL. 1934. The cytology of the abscission zone in *Mercurialis annua*. *Bull. Torrey Bot. Club* 61: 279-289.

THE EFFECT OF INDOLE-3-ACETIC ACID ON THE DRY WEIGHT OF *CHLORELLA PYRENOIDOSA*¹

Melvin Amos Brannon and Harold Melvin Sell

THIS PAPER reports the results of a study of the effect of indole-3-acetic acid on the dry weight of *Chlorella pyrenoidosa*. The culture used in this study was obtained from the department of botany at the University of Wisconsin. It was reported to be a duplicate of the culture supplied Lilly and Leonian (1941) for their study of "Some factors affecting the dry weight of *Chlorella vulgaris*."² It was labelled *Chlorella pyrenoidosa*.

METHODS.—The nutrient medium employed in this study was the same medium used by Brannon and Bartsch (1939). It consisted of 1.00 g. potassium nitrate, 0.50 g. calcium sulfate, 0.50 g. magnesium sulfate, 0.25 g. tri-basic calcium phosphate, 0.01 g. ferric chloride, and 1,000 ml. distilled water. The tri-basic calcium phosphate was first dissolved separately in 100 ml. of distilled water, and the other salts were dissolved in 900 ml. of distilled water. The solutions were then boiled, filtered, mixed and supplied with sufficient distilled water to restore the volume to 1,000 ml.

Indole-3-acetic acid in concentrations of 10 ppm. and 20 ppm. was used as growth substance in this investigation.

After adjusting the pH of the nutrient solution to 5.75–6.00, aliquots of 250 ml. were poured into 500 ml. Pyrex Erlenmeyer flasks and autoclaved for one hour at 15 pounds pressure.

The inoculum was cultured in 250 ml. of the nutrient solution for 14 days. It contained approximately 5×10^6 cells per ml. at the time of inoculating the cultures used in this work.

The cultures were grouped in series of six flasks, two containing controls and four containing indole-3-acetic acid in 10 ppm. or 20 ppm. concentration. Because experiments indicated that optimum growth developed from large inocula it was deemed well to use 1 ml. inoculum having approximately 5×10^6 cells in each flask.

The inoculations were made in a chamber having filtered air.

The series of 6 flasks was then placed in a room supplied with north light and a temperature of 28°C. The cultures were agitated for ten minutes

twice each day in order to distribute the cells in the medium and diminish agglutination. At the expiration of 30 days of incubation, the cells were harvested and the dry weights determined.

During incubation the controls developed more color and turbidity in the first 72 hours than did the indole-3-acetic acid cultures. At that period the controls and indole-3-acetic acid cultures were uniform in color and turbidity. At the conclusion of the 30-day period their respective appearances are shown in figure 1.

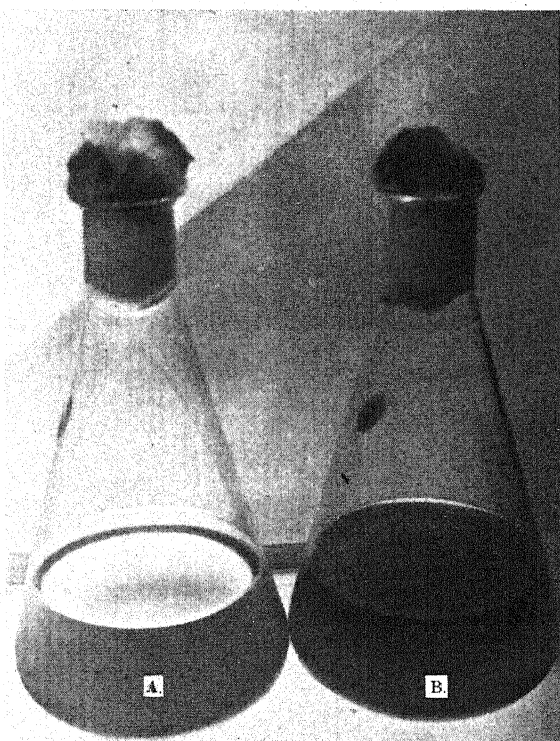


Fig. 1. Cultures of *Chlorella pyrenoidosa* after 30 days incubation at 28°C. in a room supplied with north light.
Flask A Control Flask B....Indole-3-acetic acid 10 ppm.

Dry weight	20.6 mg.	92.3 mg.
Relative dry weight	1 mg.	4.4 mg.

The dry weights of the algae were determined by centrifuging the cultures in a 250 ml. bottle at 1,500 r.p.m. for 15 minutes and then filtering the algae on dry, tared, sintered glass crucibles having a porosity of 5–10 microns. The last traces of algae from the bottles were transferred quantitatively to the crucibles by means of distilled water from a wash bottle. The crucibles were dried for 4 hours in a vacuum oven over phosphorus pentoxide at 70°C. and 4

¹ Received for publication January 27, 1945.

The work reported in this paper was done in the laboratories of the University of Florida. Grateful acknowledgment is made of the many courtesies received from Dr. J. Speed Rogers and Dr. W. R. Carroll. They provided many facilities and much equipment. The junior author made valuable suggestions as a biochemist. He recrystallized the indole-3-acetic acid, made the pH readings, and did the precision work of determining the dry weights of the cultures reported in this study. His present address is Michigan State College of Agriculture and Applied Science, Lansing, Michigan.

² Lilly and Leonian did not obtain, as they supposed, the strain of *Chlorella vulgaris* used by Brannon and Bartsch (1939), but were given a strain which turned out to be, in all probability, *Chlorella pyrenoidosa*.

TABLE 1. Showing effect of indole-3-acetic acid on dry weight of *Chlorella pyrenoidosa* measured in milligrams of dry weight of cells produced in 250 ml. cultures.

Nature of culture	Number	Mean weight	Relative weight of control and indole-3-acetic acid
Series I. Control	2	22.3 mg.	
Indole-3-acetic acid (10 ppm.).....	4	99.1 mg.	1 to 4.4 mg.
Series II and			
Series III. Control	4	25.6 mg.	
Indole-3-acetic acid (20 ppm.).....	8	120.5 mg.	1 to 4.7 mg.

mm. pressure and then cooled in a desiccator over calcium chloride. The dried crucibles containing the algae were weighed on an analytical chainomatic balance. The difference between the weight of the crucible and the weight of the crucible plus the algae gave the dry weights recorded in table 1.

DISCUSSION.—The results presented in table 1 and figure 1 show that indole-3-acetic acid in concentrations of 10 ppm. and 20 ppm. was a growth substance for *Chlorella pyrenoidosa*. These results are in agreement with the positive reactions reported for the effect of indole-3-acetic acid on *Chlorella vulgaris* by Yin (1937), Brannon (1937), Pratt (1938), and Brannon and Bartsch (1939). They are in disagreement with those reported by Lilly and Leonian (1941) in their study of "Some factors affecting the dry weight of *Chlorella vulgaris*." In view of the fact that they used the same *Chlorella* supplied in duplicate for this study this discrepancy can not be charged to the use of different species of *Chlorella*, or to a different strain of the same species.

Among the factors which might cause wide discrepancies in growth substance investigations is the growth substance itself. In early series of cultures in this study variations occurred in the indole-3-acetic acid flasks. These variations were pronounced and persistent. This problem was discussed with a biochemist who suggested that the indole-3-acetic acid had deteriorated. He stated that indole-3-acetic acid stored in an amber bottle had been known to undergo change and only a third of the original weight was recovered in purification. Following this suggestion the indole-3-acetic acid used in further investigations in this study was purified. It was recrystallized from U.S.P. chloroform to constant melting point of 165°C. When this purified indole-

3-acetic acid was used the variation in the indole-3-acetic acid cultures did not occur. Uniformly positive results were secured. This proved that defective indole-3-acetic acid was the limiting factor in the previous tests which were discarded on account of pronounced and persistent variations. Positive results were obtained in every series of this study in which recrystallized indole-3-acetic acid was employed as the growth substance.

SUMMARY

The effect of indole-3-acetic acid on the dry weight of *Chlorella pyrenoidosa* has been investigated.

Recrystallized indole-3-acetic acid (mp 165°C.) in concentrations of 10 ppm. and 20 ppm. was applied to cultures of *Chlorella pyrenoidosa* in inorganic, nutrient media. It stimulated the growth of the algae, and the dry weight of the cells was increased more than four fold.

UNIVERSITY OF FLORIDA,
GAINESVILLE, FLORIDA

LITERATURE CITED

- BRANNON, M. A. 1937. Algae and growth substances. *Science* 86:353-354.
 —, AND A. F. BARTSCH. 1939. Influence of growth substances on growth and cell division in green algae. *Amer. Jour. Bot.* 26:271-279.
 LILLY, VIRGIL GREENE, AND LEON H. LEONIAN. 1941. Some factors affecting the dry weight of *Chlorella vulgaris*. *Amer. Jour. Bot.* 28:569-572.
 PRATT, R. 1938. The influence of auxins on the growth of *Chlorella vulgaris*. *Amer. Jour. Bot.* 25:498-501.
 YIN, H. C. 1937. Effect of auxin on *Chlorella vulgaris*. *Proc. National Acad. Sci. (U.S.A.)* 23:174-176.

A MORPHOLOGIC STUDY OF THE GENUS *MONOBLEPHARELLA*¹

Martha E. Springer

THE MONOBLEPHARIDALES is an order which has been of special interest to investigators of the aquatic Phycomycetes since Cornu published his discovery of *Monoblepharis* in 1871. Its distinctive type of sexual reproduction, in which a large, nonflagellate and nearly motionless egg is fertilized by a small uniflagellate sperm, has been found in no other group of fungi. This has led to much critical study and speculation as to its origin and relationships.

The genus *Monoblepharella*, a member of the Monoblepharidales, was established by F. K. Sparrow (1940), based on a fungus recovered from soil collected by W. R. Taylor in Trinidad in 1939. Because of its obviously close relationship to species of *Monoblepharis*, the organism was first described as a member of this genus (Sparrow, 1939), but after more consideration and study it was segregated (Sparrow, 1940) into a genus of its own under the binomial *Monoblepharella Taylori* Sparrow. This change was justified by the presence in *Monoblepharella* of a motile zygote propelled by the persistent flagellum of the male gamete and also by certain other distinctive morphologic features. Although what was apparently the same fungus appeared in three other soil samples collected by W. R. Taylor, isolates from these were not critically studied, particular attention being given only to the type strain.

A second species of the genus, *M. mexicana* Shanor, was recovered from three soil samples collected in Mexico in 1941 (Shanor, 1942). Other isolates of *Monoblepharella* have been obtained from soils collected by C. D. LaRue in Mexico, Central America, and South America in 1940, and from California soils collected by the author in 1942.

The eleven isolates available for the present investigation have included both of these originally described by Sparrow and Shanor, and also a number of others from localities distributed widely in the Western Hemisphere. It has seemed advisable, therefore, to make a thorough study of these isolates, to observe possible variations, and to confirm and supplement certain points in the developmental history and morphology of members of the genus. As a result of this study two new species have been described (Springer, 1945).

MATERIALS AND METHODS.—The material available consisted of cultures started from the Taylor collections by Sparrow, a culture of *M. mexicana* furnished by Shanor, and soil samples from the other

collections. All of the soils were collected in clean new boxes or envelopes and sealed. In the laboratory they were placed in jars of sterile distilled water containing pieces of sterilized hemp seed. By the occasional addition of fresh hemp seed bait, the original cultures have been kept viable over a period of several years. Of the eleven isolates involved, one has produced sporangia but no sex organs and has not been assigned to a species. The other ten were identified as belonging to four species, *M. Taylori* Sparrow, *M. mexicana* Shanor, *M. elongata* Springer, and *M. Laruei* Springer.

Because of its extreme delicacy and extraordinarily slow rate of growth, the mycelium of *Monoblepharella* requires special handling. During the period of one or more months elapsing before it forms a colony of appreciable size in the gross culture, other filamentous fungi, such as *Allomyces*, *Pythium*, and members of the Saprolegniales and Fungi Imperfecti, may flourish and die down. Although *Monoblepharella* can be grown on artificial media (Sparrow, 1943) the hyphae grow so much more slowly than those of the fungi with which it is associated that it cannot easily be isolated. It was found possible, however, to segregate *Monoblepharella* from other Phycomycetes by repeatedly multiplying, washing, and selecting isolated colonies. The contaminating Fungi Imperfecti were found to grow best only at the surface of the water, and since *Monoblepharella* will thrive at a depth of several inches, it was separated from these fungi by growing it in deep water. Then by the use of shallow water cultures it was possible to grow and multiply the various isolates in a period of one or two weeks instead of one or two months. None of the cultures was entirely freed of bacteria.

A large number of the very small zoospores and zoosporangia were isolated in an unsuccessful attempt to obtain single spore cultures. Equivalent cultures could be started in certain instances by isolating a single heavily nodulated element of the mycelium and placing it in a drop of water with a very small bit of hemp seed. Up to the present time this method has been successful in only two instances, in the type isolates of *M. Taylori* and *M. elongata*.

All of the isolates have been cultivated under similar environmental conditions, and the examinations of various stages in their development and morphology are therefore comparable. The formation of zoosporangia in large numbers may be induced by starving the mycelium by removing it from the bait and placing it in fresh water. Both Sparrow (1940, 1943) and Shanor (1942) mentioned that at room temperature (specified by Sparrow at 21°C.) more zoosporangia are produced, while at 30°C. sex organs are abundantly formed. Neither author, however, has reported any attempt to deter-

¹ Received for publication February 1, 1945.

This paper is part of a dissertation submitted in partial fulfillment of the requirement for the degree of Doctor of Philosophy in the University of Michigan.

The writer wishes to express her appreciation to Dr. W. R. Taylor and Dr. C. D. LaRue for furnishing most of the soil collections, to Dr. Leland Shanor for the culture of *Monoblepharella mexicana*, and especially to Dr. F. K. Sparrow for providing several of the cultures, and for his direction and helpful suggestions during the investigation and the preparation of this paper.

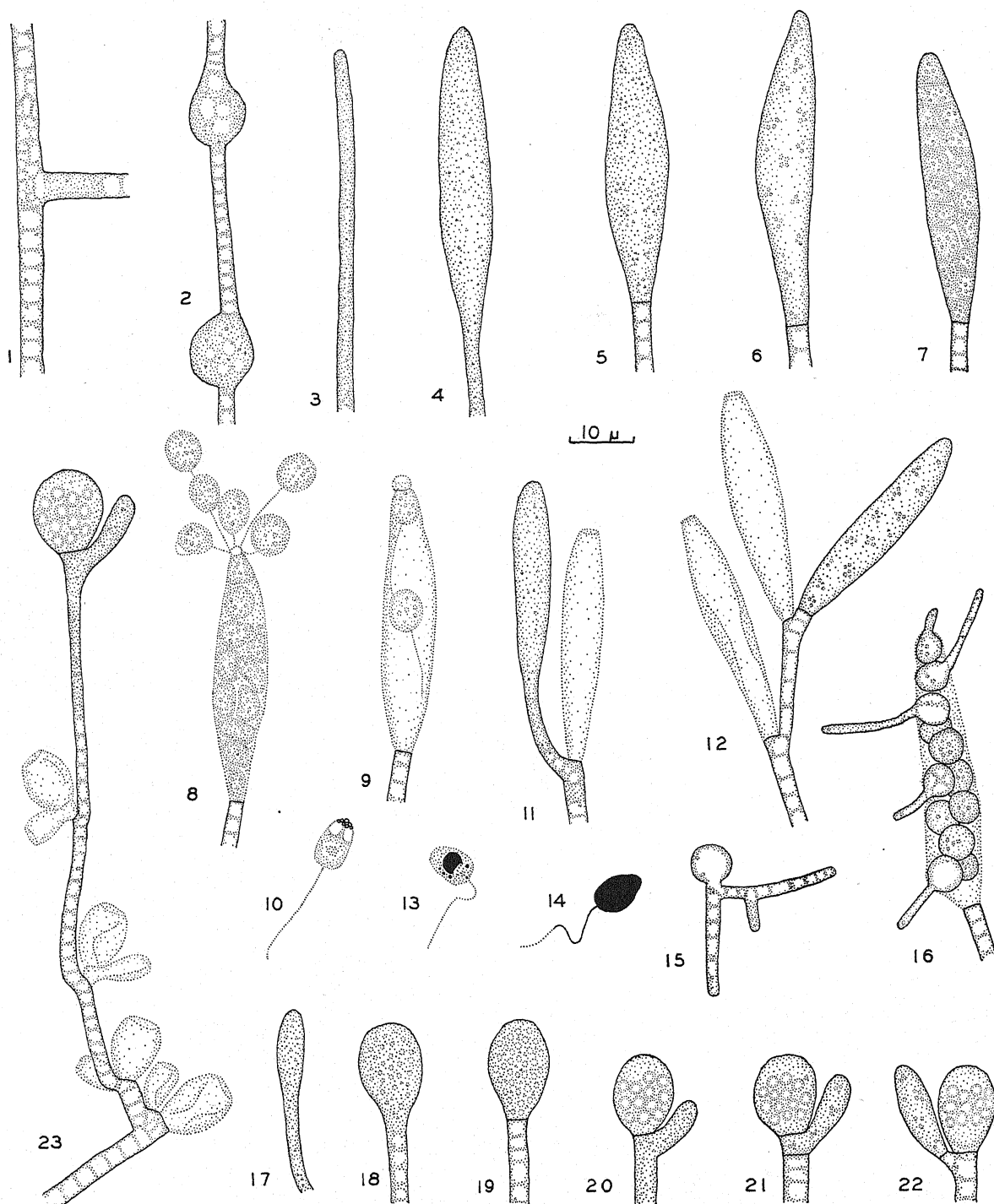


Fig. 1-23. Camera lucida drawings of *Monoblepharella Taylori*— $\times 1000$.—Fig. 1. Portion of mycelium showing vacuolated protoplasm (T₃).—Fig. 2. Mycelium with nodules (T₁).—Fig. 3-6. Sporangia in successive stages of development (T₃).—Fig. 7. Mature sporangium (T₃).—Fig. 8-9. Stages of emergence from sporangium shown in figure 7.—Fig. 10. Zoospore (T₁).—Fig. 11-12. Stages in development of secondary sporangia (T₁).—Fig. 13. Cytological preparation of zoospore, showing primarily the dark staining nuclear cap, and the granule, the blepharoplast, at the base of the flagellum (T₂).—Fig. 14. Zoospore stained to show whip-lash type of flagellum (T₂). Note tip of flagellum.—Fig. 15. Germinating zoospore (T₂).—Fig. 16. Zoospores germinating within a sporangium (T₁).—Fig. 17-19. Stages in development of oogonial rudiment (T₁).—Fig. 20. Formation of a sub-oogonial branch (T₁).—Fig. 21. Oogonium and hypogynous antheridium (T₁).—Fig. 22. Oogonium and terminal antheridium (T₁).—Fig. 23. Branch bearing several pairs of sex organs, mostly empty (T₂).

mine ranges of temperature or the optimum temperatures at which these two types of organs are developed. By placing bits of mycelium at various temperatures it was found in the present investigation that sporangia were produced in all isolates at temperatures ranging from 13–32°C., and in four of the isolates of *M. Taylora* up to 37°. The optimum for all isolates is between 21° and 31°C. Within this range sporangia will be produced abundantly in three or four hours, but both above and below these limits fewer are developed and more time is required for their production. At 13° or 37°C., for example, probably no sporangia will be formed in less than seven to ten hours.

Although sporangia are formed in abundance when the mycelium is suddenly starved, sex organs are formed in abundance only when the mycelium is left undisturbed for several days at temperatures ranging from 26° to 32°C. Frequently a sudden change in conditions, such as the removal of a piece of mycelium to fresh water in order to study the sex organs, stimulates the production of sporangia, and it is possible to have large numbers of both types of organs produced simultaneously. If the change of conditions occurs prior to the development of the cross wall, an oogonial or antheridial rudiment may cease its development and become transformed into a sporangium.

MORPHOLOGY.—The isolates studied exhibit little variation in their vegetative growth. Differences in the nonsexual reproductive organs relate mainly to shape and size, and overlappings and intergradations of these characters are frequent. It is in the sequence of development, the resulting arrangement, and the size and shape of the sex organs that most of the variations between species are apparent. The method of formation of the different structures and the sequence of changes in the cytoplasm are almost identical. A description of fertilization and the emergence and motility of the zygote for one species will apply equally well to the other three. Furthermore, differences in the measurements of the antherozoids, zygotes, and oospores of the four species are so slight as to fall well within the range of experimental error. A thorough discussion, therefore, of the morphology will be given only for *Monoblepharella Taylora*, the type species of the genus, after which the other species will be discussed more briefly, and their outstanding characteristics pointed out.

Monoblepharella Taylora Sparrow

- Isolates: T₁. Collected by W. R. Taylor, Trinidad, B. W. I. Type.
T₂. Collected by M. E. Springer, California.
T₃. Collected by W. R. Taylor, Panama.
T₄. Collected by C. D. LaRue, Nicaragua.
T₅. Collected by W. R. Taylor, Trinidad, B. W. I.

T₆. Collected by W. R. Taylor, Panama, Canal Zone.

Although Sparrow (1940) made a very careful and accurate report of this species, especially of the sexual apparatus, fertilization, and emergence of the zygote, it was based upon only one isolate and a limited number of observations. The following discussion, therefore, serves to confirm his observations and to supplement them particularly with respect to the asexual and developmental stages and the limits of size and shape of the organs.

Mycelium.—The mycelia of the various species of *Monoblepharella* are so distinctive that members of the genus may be identified in vegetative condition alone. Because it develops very slowly, the characteristic growth becomes conspicuous in a water culture only after a period of one or more months. By then, the colony may reach a diameter of 25 mm. and can be recognized as a lustrous pearly gray halo around the substratum. The hyphae are nonseptate and so delicate that they are distinguishable even from those of other members of the Monoblepharidales, all of which possess the same type of vacuolation.

So far as could be determined, there is no truly differentiated holdfast system such as is found in *Monoblepharis* and many other filamentous fungi. The hyphae at the base, however, are larger and more frequently branched than those on the periphery. Since the hyphae are so delicate that they are able to penetrate almost any part of the bait, it is possible that a true holdfast is not necessary, the ramifying hyphae sufficing both for attachment and food absorption. Branches of vegetative hyphae are almost exactly at right angles to the main axis. The edge of a colony is composed of a large number of straight unbranched hyphae which radiate from the substratum. These filaments are exceedingly long and tenuous, with slight variations in diameter throughout their length. The tips are rounded somewhat but not noticeably tapering. Usually there is no special thickening at the point of origin of the branches, although they may occasionally arise from a triangular expansion or from a well defined nodule. The hyphae vary in diameter from 1 to 7.5 μ , with 2 to 3 μ being most typical. The zoosporangia and sex organs are produced on vegetative elements of average size.

The mycelium (fig. 1) has the regular scalariform or reticulate vacuolation characteristic of the Monoblepharidales. In the more delicate hyphae the vacuoles frequently lie in a single row separated by bands of cytoplasm so narrow as to give a ladder-like appearance. The arrangement of vacuoles in the wider hyphae is sometimes not so regular, but the cytoplasmic network is none the less conspicuous. There may be a little vacuolation in young or vigorously growing hyphae, and older ones may be almost empty. There are, however, always a few hyphae in cultures of all ages which maintain the

typical disposition of contents, and serve to identify the fungus.

The protoplasm in a vigorously growing hypha is extremely active, and under high magnifications its movements may easily be observed. It contains many refractive oil droplets the path of which, irregular because of the presence of so many vacuoles, may be followed along the hypha. So characteristic is this movement that a reversal of direction has been utilized successfully as an index of the presence of the delicate cross wall formed in the development of the reproductive organs.

Swellings of various shapes have been found on the mycelium of all isolates observed, but the number and size differ considerably, even in different subcultures of the same isolate. These were noticed and commented upon by Sparrow (1940) and Shanor (1942—for *M. mexicana*), the former author leaning toward the belief that they are caused by a parasitic organism, although he states that no reproductive phase has ever been observed to substantiate this suspicion. Shanor considers them, also without contributing evidence, to be normal structures belonging to the fungus itself.

The distribution of the nodules does not appear to be regular. On one piece of substratum most of the hyphae may show no trace of such swellings, while a few branches may be very heavily nodulated. The swellings vary in shape from globose or spindle-shaped (fig. 2) to large irregular masses. In an old culture the nodules may be found in what are apparently progressive stages of vacuolation, from being filled with a nearly homogeneous mass of cytoplasm to being completely empty. Those which are nonvacuolated often appear highly refractive. In more vacuolate nodules, the cytoplasm may appear frothy or scalariform. The largest nodules observed in the six isolates of *M. Taylori* ranged from $17 \times 17 \mu$ to $35 \times 20 \mu$. Most of them were much smaller. From some nodules normal or occasionally abortive branches may arise.

For the successful development of a new colony from a single element of mycelium it was found necessary to use a piece with several nodules, and it was noted in the isolates both of *M. Taylori* and *M. elongata* that the new branches arose from these swellings, apparently indicating that the nodules are reservoirs of protoplasm and are indeed normal structures.

Asexual reproduction.—The organs of asexual reproduction are elongate cylindrical or fusiform zoosporangia which produce posteriorly uniflagellate zoospores. By reason of their refractive appearance and large size as compared with the delicate mycelium, the sporangia are very conspicuous, and, as has been pointed out by Sparrow, frequently resemble elongate conidia lying free in the mycelial mass.

The sporangia develop terminally on the hyphae, primarily at the periphery of the colony. They may appear as early as four days after the establishment of a new culture and have also been observed

in cultures four or five months old. The method of development of the zoosporangium is identical in all isolates, and is similar to that of *Monoblepharis*, which has been described by Lagerheim (1900), Laibach (1927) and Sparrow (1933). Although only a very superficial study has been made of the cytology of sporangial development of *Monoblepharella*, it, too, appears to resemble *Monoblepharis*, as described by Laibach (1927). In the initial stages, the tip of a vigorously growing hypha becomes filled with very rapidly streaming, irregularly spaced and highly refractive oil droplets (fig. 3). The tip then begins to elongate and swell (fig. 4). As more droplets accumulate, their motion becomes less rapid. A stained preparation will show that at this time there are many more nuclei present than in an ordinary vegetative hypha of the same length. Their mode of origin has not been determined. In the closely related genus *Monoblepharis* no mitotic figures were observed by Laibach during sporangial development. It is not known, therefore, whether the number of nuclei has increased by migration from other parts or by nuclear division.

By the time the somewhat cylindrical sporangial rudiment has reached its full size, its contents are non-vacuolate, and practically motionless, in sharp contrast to the vacuolate attendant hypha in which the cytoplasmic streaming is still conspicuous. A basal cell wall is soon laid down (fig. 5). Meanwhile the droplets within the sporangial rudiment have started to aggregate, and regularly spaced clusters of small globules become conspicuous in the otherwise clear matrix (fig. 6). In the delimitation of the zoospores the cytoplasm becomes separated into a number of nearly equal parts, each containing a cluster of globules and, as may be seen in a stained preparation, one nucleus. Depending upon the diameter of the sporangium, the spores are formed in one or more rows. The rudiments of the zoospores are at first angular. They soon draw apart slightly, become somewhat more rounded, and are then apparently mature (fig. 7).

Discharge of the zoospores is initiated by the deliquescence of the apex of the sporangium to form a small pore. In escaping from the sporangium, a part of the thin clear protoplasm of the first zoospore oozes through the opening and swells up outside. The globules then gradually move forward, and the rest of the body emerges. The oil globules at this time are irregularly spaced throughout the spore. After a momentary hesitation the spore moves away but is stopped by its flagellum, which is not yet free from the sporangium. The body of the spore may then undergo slight amoeboid changes of shape. Since the first three to six spores usually emerge in very rapid succession, they form a cluster attached to the sporangium by their flagella (fig. 8). In a short time, perhaps a minute after its emergence, the first spore will struggle to free its flagellum, either by a number of short jerks or by several violent tugs. As the spore revolves, it may be seen that the globules, up to twelve or fif-

teen in number, are lining the front of what now appears to be a hollow ovoid rather than a spherical body. After some minutes the tip of the flagellum is suddenly freed from the sporangium. The spore remains motionless for an instant and then darts away, soon to be followed by the others. The rapid emergence of the first spores has apparently lowered the pressure within the sporangium, and the others are more leisurely about escaping. They approach the opening in amoeboid fashion and slowly ooze out one at a time. The flagella of those emerging or those about to emerge are easily visible (fig. 9).

Although the size of the sporangium does not determine the size of the zoospore, it does limit the number produced. Nor are the differences in number a constant character of individual isolates, for the smallest as well as the largest number of zoospores in a single sporangium, eight and thirty-six respectively, were observed in sporangia of the same isolate, *T*₅. The numbers most frequently encountered are fourteen to twenty.

Secondary sporangia are produced by sympodial branching of the hypha. A swelling appears immediately below the cross wall of the terminal sporangium and elongates into a branch which produces a sporangium at its tip (fig. 11). As a result of this renewed growth the sporangium which was originally terminal appears lateral. Ordinarily secondary sporangia are not formed very close together, but sometimes so many develop in rapid succession that they form a cluster at the end of the hypha (fig. 12).

Within the species there is a wide variation in the shape and size of the sporangia, so much so that their use as a criterion for distinguishing species is not satisfactory. They are symmetrical or slightly falcate, narrowly siliquiform, fusiform, or cylindrical, with rounded apex, tapering at the base to a diameter about equal to that of the attendant hypha. Lateral protuberances on the sporangial wall have been noted in all isolates, but they do not occur with the same frequency in all species. In the isolates of *Monoblepharella Taylori* they occur only rarely but when present may be so pronounced as to form lobes. They function as exit tubes for the

zoospores, supplementing the sporangial apex, which is the typical place of emergence.

The sporangia of the first five isolates of this species, *T*₁–*T*₅, are 18–84 μ in length and 5–16 μ in width. Sporangia of *T*₆, however, are more variable in length (20–115 μ), with a width of 6–14 μ . With a mean ratio of length to diameter of 5.9:1, they approach the average ratio for the species, 5.5:1 (diagram 1).

The zoospore (fig. 10), although smaller, is similar to that of *Monoblepharis*. From the spherical or slightly oval shape it possesses directly after emergence, it changes while in motion to become more cylindrical or ovoid. The strongly refractive globules at the somewhat pointed forward end now appear to be almost separated from the rest of the spore. The anterior end stands out in conspicuous contrast to the evenly granular material lining most of the body, from which it is separated by a space which is clear except for a central strand of cytoplasm. Sometimes in the living zoospore a slightly darker area, presumably the nuclear cap, can be distinguished. At the point of attachment of the single posterior flagellum a very small refractive body is visible.

Cytological preparations of the zoospores were made according to two methods. The first is that of Cotner (1930), and was used to demonstrate the nuclear apparatus. The internal structure of the spore (fig. 13) was found to be similar to that already noted in the Blastocladiales and Monoblepharidales (Cotner, 1930; Sparrow, 1933). There is a darkly-stained nuclear cap in the center of the cell. Faint lines can be distinguished extending from the sides of this structure back to a well defined basal granule, or blepharoplast, located at the point of attachment of the flagellum. The conical area thus defined is less strongly stained than is the surrounding cytoplasm. The second method, employed to demonstrate the type of flagellum, utilized the simpler of the two techniques described by Couch (1941). With high magnification the whip-lash character of the flagellum was made clearly distinguishable (fig. 14). The extremely fine projection extended 3–6 μ beyond the thicker main part of the flagellum.

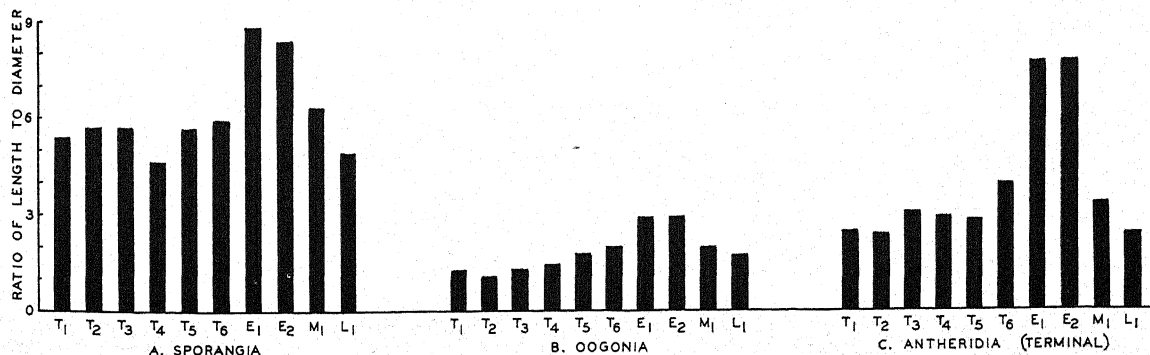


Diagram 1. Mean ratios of length to maximum diameter of one hundred of each of the reproductive organs of ten isolates of *Monoblepharella* spp. (*M. Taylori* *T*₁, *T*₂, *T*₃, *T*₄, *T*₅, *T*₆; *M. elongata* *E*₁, *E*₂; *M. mexicana* *M*₁; *M. Laruei* *L*₁).

Measurements of size are made difficult by the changes in shape undergone by the zoospores. They are spherical when they emerge from the sporangium but become ovoid while actively motile. When they slow down they become oval or amoeboid in shape. Swimming spores are 7–10 μ long and 4–6 μ wide. Instances have been observed in which giant or dwarf spores have been produced in the same sporangium by abnormal cleavage of the protoplasm. In general the flagella are two to three times the length of the body of the spore, the majority being 20–29 μ .

The zoospore in open water swims with a smooth gliding movement, inscribing rather large circles. Occasionally, when becoming entangled in some of the branches of the mycelium, it undergoes a violent shaking motion which extends through the body of the spore and the flagellum. More often, however, its movement remains even. At intervals it may remain motionless except for a slow lashing of the flagellum. The period of motility varies, in some instances lasting at least a half hour, in others only a few minutes. After a period of swimming the spores may rest or show slight amoeboid movement and then once more swim away. This may happen several times before they finally come to rest and encyst.

Within a quarter or half hour after the zoospore encysts a short germ tube appears. This elongates and produces branches at right angles to establish the vacuolated mycelium typical of *Monoblepharella* (fig. 15). Rarely there may be two germ tubes.

Sometimes, especially in heavily contaminated cultures, the first zoospores to emerge may clog the pore so that few or none are able to escape. They may then germinate within the sporangium, the germ tubes penetrating the sporangial wall (fig. 16). In other instances environmental conditions may change and thereby stop the development of the sporangium before the zoospores are delimited. The sporangium may then revert to vegetative growth.

Sexual reproduction.—The sex organs of *Monoblepharella* are oogonia and antheridia which are typically associated in pairs and are produced alternately on the same hypha. Sex organs are produced in all isolates of *M. Taylori* at all temperatures between 26 and 32°C. Development of the oogonium is initiated with the collection in the tip of a vigorously growing hypha of highly refractive and rapidly streaming oil droplets (fig. 17). The tip gradually swells until it is almost spherical (fig. 18). Meanwhile the droplets coalesce until finally the entire swollen portion is filled with small oil globules. About this time the cross wall is formed (fig. 19), delimiting the oogonial rudiment a short distance back of the swelling, thus leaving a short narrow neck. The oil droplets continue to fuse and at maturity there are within the egg a number of very large and conspicuous globules which arrange themselves so compactly in a band in the center as to leave a zone of very clear ooplasm at the tip and

at the base of the oogonium. From seven to thirty globules have been observed in typical eggs. The mature oogonium is 10–22 μ long by 7–16 μ in maximum diameter. At maturity the egg may round up and withdraw slightly from the base of the oogonium, but this condition is more often observed in those oogonia with rather long cylindrical bases than in those in which the base is broadly truncate. If several eggs are to be produced, they can be distinguished about the time that the globules have attained their full size. Sparrow (1940) records one instance in which six eggs were formed in an exceptionally large oogonium. The greatest number observed in this study was five. The eggs vary greatly in size and in the number of globules they contain.

Soon after the cross wall is formed at the base of the oogonium, a small protrusion appears immediately below it (fig. 20). This may be an integral part of an hypogynous antheridium (fig. 21) as described by Sparrow, or it may develop into a short branch on which an antheridium develops terminally (fig. 22). In either case the stages of development of the antheridium are like those of the sporangium. In the hypogynous type the cylindrical hyphal portion is 2–12 μ in length and the beak-like lateral outgrowth is 4–15 μ long by 4–7 μ wide. The terminal antheridia are 8–25 μ long by 4–8 μ wide. The method of formation of the antherozoids also is like that of the zoospores. By transverse cleavage, two to seven antherozoid rudiments are delimited, each containing one cluster of small globules. The segments of protoplasm round up and the antherozoids are ready to emerge.

A lateral branch may arise just below the mature antheridium and, after growing for a distance several times the length of the antheridium, develop another oogonium. Because of sympodial branching, the older sex organs, usually empty, appear to be lateral (fig. 23), and the hypha seems to follow a very irregular course. In old or heavily contaminated cultures the pairs of sex organs may be formed in very close proximity and be clustered at the tip of the hypha. Rarely more than one antheridium may be cut off on a hypha below an oogonium, or several oogonia may be produced consecutively, without the alternate development of antheridia.

The antherozoids escape through a pore at the apex of the antheridium or the antheridial beak. They are much like the zoospores but smaller, 5–6 μ long by 3–4 μ wide, and are much more amoeboid, during both discharge and rest or creeping periods. No rhythmic sequence of discharge of antherozoids and maturing of the eggs could be determined. In some instances the two are simultaneous, but frequently the antherozoids are discharged before the egg is mature, or the oogonium is emptied before the maturation of the antherozoids. The place of origin of the antherozoid was not determined in most observed cases of fertilization.

The process of fertilization was accurately described by Sparrow (1940). It was observed in the

present material that when an antherozoid reaches a mature oogonium, it becomes amoeboid, creeping over the surface of the oogonial apex. Its single flagellum protrudes motionless almost at right angles away from it, or sometimes waves slowly back and forth. No receptive papilla has been noted on the oogonium, and it is not until the antherozoid becomes quiescent at the tip that it becomes possible to locate precisely the oogonial orifice. The body of the antherozoid sinks slowly into the ooplasm. Not all of the male gamete is engulfed by the egg, a part remaining more or less flattened on the surface of the oogonium (fig. 24). Suddenly the clear ooplasm begins to flow out through the oogonial orifice (fig. 25), and it is now impossible to distinguish the protoplasm of the antherozoid from that of the egg. The flagellum still extends straight and motionless in the water. After some of the clear watery cytoplasm has emerged, the large globules pass through the orifice, a few at a time (fig. 26). They flow back and forth with the movement of the emerged portion of the zygote. Within a few minutes the globules have all passed out of the oogonium; the remaining cytoplasm oozes out; and the zygote has completed emergence. The persistent flagellum of the antherozoid has by this time moved around so that it is lateral to the oogonium. The emerged zygote is somewhat ovoid at first, but almost immediately rounds up. After a very short period of quiescence, it begins to rock back and forth, often sending out clear pseudopodia and retracting them. With a jerk it suddenly moves away from the oogonium, hesitates momentarily, and with a very characteristic rolling, halting, and rocking movement swims away.

The moving zygote (fig. 27) is spherical or broadly ovoid, 10–13 μ long by 8–10 μ wide. It progresses so slowly that it is frequently possible to see the moving posterior flagellum. The flagellum may be visible throughout the entire period of fertilization, emergence, and the early swimming stages, thus giving definite evidence that it is indeed, as Sparrow contended, the flagellum of the antherozoid which propels the zygote. As the zygote rolls and turns, the globules are easily distinguishable and seem to form a compact peripheral layer lining all except the broadly conical anterior end of the zygote. Motility of the zygote may continue for at least half an hour, during which time it may stop to rest or to undergo strong amoeboid crawling (fig. 28) before swimming off again. Finally it becomes motionless. The flagellum is no longer visible; according to Sparrow, it appears to be condensed into a small droplet and is then absorbed. The encysted zygote (fig. 29) soon becomes surrounded by a thickened wall, smooth and light brown in color. The large globules may persist in the oospore, or they may be absorbed, in which case there are small refractive droplets throughout the protoplasm. Typically the oospore (fig. 30) is formed free in the water, and at some distance from the oogonium. It is spherical and 9–13 μ in diame-

ter. Under poor environmental conditions it may be formed at the oogonial orifice or may be retained within or partly within the oogonium.

Germination of the oospores may be obtained by adding fresh water and bait to an old culture or to dried spores. It is not known how long the oospore will remain viable. Sparrow was able to obtain germination from fully mature oospores dried for three weeks. In the present investigation good growth of the isolate T₁ was obtained in a culture started with a part of the original soil sample four years after the date of collection. Germination occurs by the formation of a small pore in the oospore wall, through which the hypha emerges. This elongates and branches (fig. 31), re-establishing the plant. The vacuolate condition typical of the mature mycelium is apparent from the first.

Although the six isolates identified as *M. Taylori* are very similar, no two are morphologically identical. The differences in the sex organs relate almost exclusively to the comparative numbers of terminal and hypogynous antheridia and to slight variations of shape and size of the parts. In isolates T₁ and T₃ hypogynous antheridia are more common than the terminal ones; the reverse is true in isolates T₂ and T₄, and the two types occur with almost equal frequency in cultures of T₅ and T₆. The antheridia are generally shortest in isolates T₁ and T₂, and progressively longer as one proceeds from T₃ to T₆. The oogonia in T₁ to T₄ are very similar to one another in appearance, many being almost spherical. In T₅ they are somewhat more elongate and narrow, while in T₆ they are of the same general shape as those of T₅, but larger. There are, however, so many intergradations in shape and size of both oogonia and antheridia that it is impossible by the examination of only a few organs to identify the particular isolate on which they have developed. It is only by a comparison of a large number of structures that the slight differences become noticeable.

Although there is rarely more than one egg produced in an oogonium in any of the isolates, the formation of two to four is of slightly more frequent occurrence in isolate T₆ than in the others, and is probably correlated somewhat with the larger size of its oogonia.

Monoblepharella elongata Springer

Isolates: E₁. Collected by C. D. LaRue, Mexico. Type.

E₂. Collected by M. Springer, California.

Mycelium.—The mycelium, although like that of *M. Taylori* in other characteristics, differs from that of all other species in its degree of nodulation. Sometimes nodules are very infrequent, but more often they are abundant and very large, attaining a diameter of 50 μ . Such nodulated hyphae superficially resemble the mycelium and sporangia of a species of *Pythiogeton*. The excessive nodulation may result in a difference in the macroscopic ap-

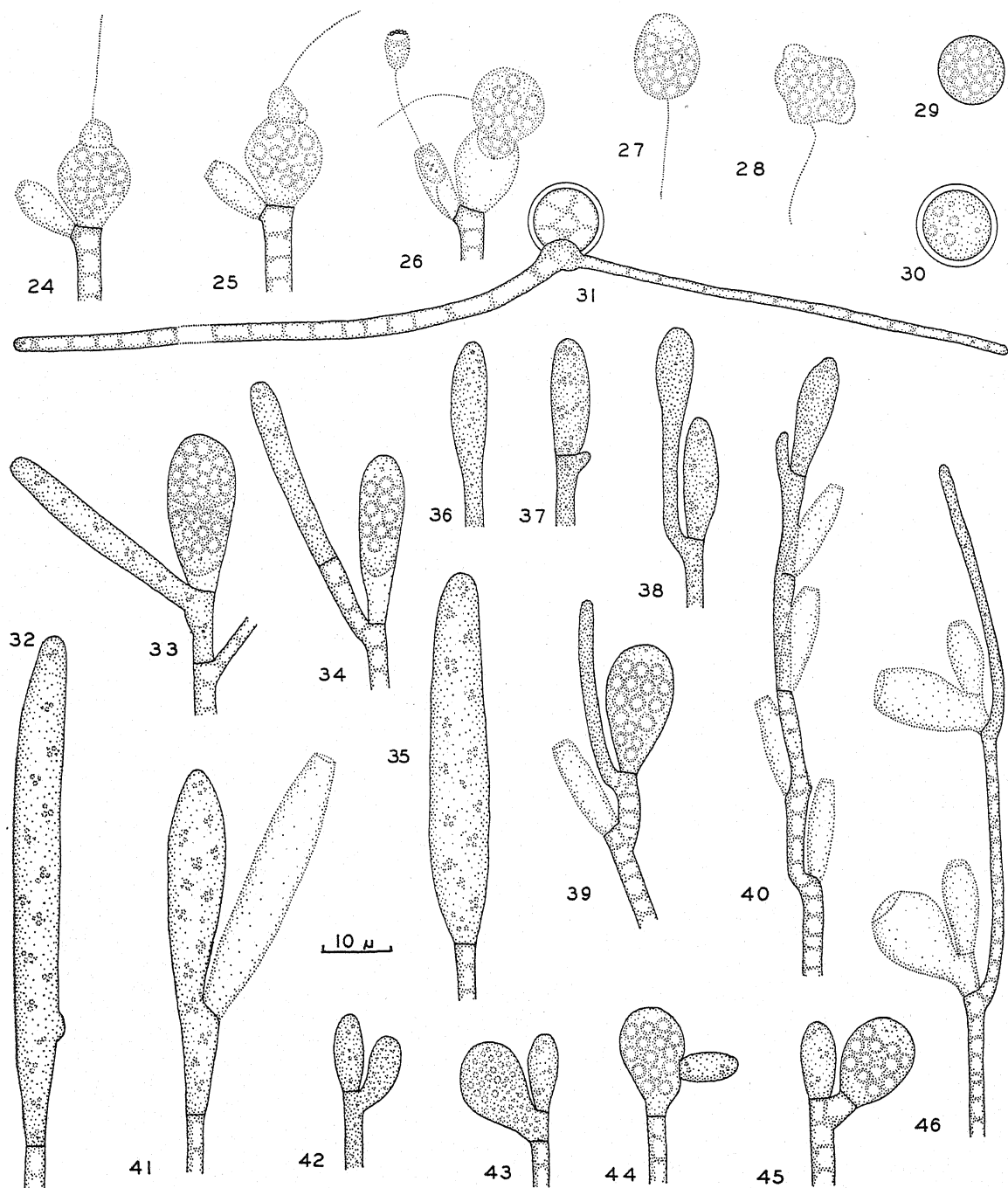


Fig. 24-46. Camera lucida drawings of *M. Taylori*, *M. elongata*, *M. Laruei*, *M. mexicana*. $\times 1000$.—Fig. 24-31. *M. Taylori*.—Fig. 24. Fertilization (T_2).—Fig. 25. Early stage in emergence of zygote (T_2).—Fig. 26. Antherozoids and zygotes emerging simultaneously (T_2).—Fig. 27. Zygote (T_2).—Fig. 28. Zygote undergoing amoeboid movement (T_2).—Fig. 29. Encysted zygote (T_2).—Fig. 30. Oospore (T_1).—Fig. 31. Germinating oospore (T_1).—Fig. 32-35. *M. elongata* (E_1).—Fig. 32. Typical sporangium.—Fig. 33. Oogonium with two eggs; antheridium hypogynous.—Fig. 34. Oogonium and terminal antheridium.—Fig. 35-40. *M. mexicana*.—Fig. 35. Typical sporangium.—Fig. 36-37. Stages in development of antheridium.—Fig. 38. Antheridium and early stage in formation of oogonium.—Fig. 39. Oogonium and empty antheridium.—Fig. 40. Branch bearing only antheridia.—Fig. 41-45. *M. Laruei*.—Fig. 41. Basipetally formed secondary sporangia subtending empty primary organ.—Fig. 42. Terminal antheridium and early stage in development of the oogonium.—Fig. 43. Later stage in development of oogonium; antheridium now epigynous.—Fig. 44. Oogonium tilted to appear terminal on hypha; antheridium now lateral.—Fig. 45. Antheridium and terminal oogonium.—Fig. 46. Branch with two pairs of empty sex organs.

pearance of the fungus, causing the growth to appear less delicate, more cottony, and more compact.

Asexual reproduction.—The sporangia differ from those of the other species in two main respects. They are the longest and comparatively the narrowest studied, measuring $38\text{--}122\ \mu$ long by $4\text{--}14\ \mu$ wide, with the mean ratio of length to diameter, 8.7:1, approximately one and one-half times that of *M. Taylori*. Because the zoospores are usually uniseriate, the number is not greater than that in the shorter and wider sporangia of the other species, where they may be formed in several rows. The sporangia (fig. 32) of *M. elongata* bear typically at least one lateral papilla, a condition rare in all of the other species.

Sexual reproduction.—Although sex organs of *M. elongata* have developed at temperatures of $26\text{--}32^\circ\text{C}$., they are most abundantly produced at about $26\text{--}27^\circ\text{C}$. The method of development and the resulting arrangement of sex organs are like those of *M. Taylori*, from which they differ chiefly by reason of their elongate appearance, and the typical formation of more than one egg in the oogonium. The mature oogonium is $17\text{--}35\ \mu$ long by $7\text{--}12\ \mu$ in diameter. In about one-half of the oogonia one egg is produced; in the others there are usually two or three, although as many as eight have been observed. Both terminal and hypogynous antheridia may be produced. The terminal ones (fig. 34) are $20\text{--}45\ \mu$ long by $3\text{--}5\ \mu$ wide—little broader than the attendant hyphae. The hypogynous antheridium (fig. 33) consists of a cylindrical section of the suboogonial hypha $2\text{--}19\ \mu$ long, and a protruding beak $10\text{--}35\ \mu$ long by $3\text{--}5\ \mu$ wide. Both types may have lateral papillate outgrowths, but these have not been observed to function as exit pores, as do similar projections on the sporangia. Four to seven antherozoids may be delimited.

Fertilization and the emergence of the zygote occur precisely as in *M. Taylori*. Often, however, the zygote does not emerge or, if it does, encysts at the mouth of the oogonium. In cases of non-emergence, the oospore assumes the shape of the oogonium.

Monoblepharella mexicana Shanor

Isolate: M₁. Collected by W. C. and M. M. Leavenworth, Mexico.

Mycelium.—Like that of *M. Taylori*.

Asexual reproduction.—The measurements of the length of the sporangia of *M. mexicana* (fig. 35) are intermediate between the isolates of *M. Taylori* and *M. elongata*, ranging from 23 to 92 μ , while the average width, 9.4 μ , is about the same as that of *M. Taylori*. The mean ratio of length to diameter in *M. mexicana* is 6.3:1, slightly higher than in *M. Taylori*, but much longer than in *M. elongata*. The development of secondary sporangia is typically sympodial, as in the preceding species, but occasionally may be basipetal.

Sexual reproduction.—More difficulties have arisen in attempts to induce the formation of sex

organs in *M. mexicana* than in any other species. These organs have been found to develop only at 30°C ., and even at this temperature they appear only sporadically. It is evident that further investigation will be necessary to determine the optimum conditions for the production of sex organs in this species.

The sequence of development of the oogonia and antheridia, as described by Shanor (1942), is exactly the reverse of that in *M. Taylori* and *M. elongata*. In the present material it was observed that the antheridium develops terminally on a hypha (fig. 36–37). When mature it is $11\text{--}25\ \mu$ long by $4\text{--}8\ \mu$ wide and bears from two to eight antherozoids. It may have the rounded apex common to other species, or it may taper very slightly apically. On the tip of a branch arising beneath the antheridial cross wall, either another antheridium or an oogonium may develop. Hyphae bearing only antheridia are common in the species (fig. 40) and occasionally branches are produced on which there are many antheridia and only a few oogonia. Typically, however, an oogonium will be formed on the lateral branch directly above the male organ (fig. 38–39). The mature oogonium is $13\text{--}25\ \mu$ long by $7\text{--}15\ \mu$ wide. It usually bears only one egg. By sympodial branching additional pairs of sex organs may be produced. The arrangement, with the male structure always slightly below the female, is a very characteristic and striking feature of this species. In old or heavily contaminated cultures, the sex organs show a strong tendency to be clustered, more so than in other species. The eggs formed under such conditions are usually abnormal, containing a few very large globules, and often encysting or disintegrating in the oogonium.

Perhaps the sequence of development of the sex organs and the frequent production of antheridia without accompanying oogonia are of advantage in ensuring frequent cross fertilization, but this cannot be stated with certainty.

Monoblepharella Laruei Springer

Isolate: L₁. Collected by C. D. LaRue, Nicaragua.

Mycelium.—Like that of *M. Taylori*.

Asexual reproduction.—It is in the formation of secondary sporangia by basipetal development that the asexual stages of *M. Laruei* are distinctive. The primary sporangium closely resembles that of *M. Taylori*. Instead of developing into a branch bearing a terminal secondary organ, the swelling below the cross wall develops directly into a geniculate sporangium, which is then delimited basally from its attendant hypha by a cross wall (fig. 41). Several sporangia may form in this manner, one below the other. Typically, as each sporangium develops, it comes to lie with its long axis in line with that of the hypha, and the first-formed sporangium now appears to be a lateral outgrowth. This method of sporangial development has been observed in *M. Laruei* in comparatively clean cultures and may be

TABLE 1. Mean measurements in microns of one hundred of each of the reproductive organs of ten isolates of *Monoblepharella*.

Isolate	Sporangia		Oogonia		Antheridia	
	Length	Width	Length	Width	Length	Width
	μ	μ	μ	μ	μ	μ
<i>M. Taylora</i>						
T ₁	49.5	9.5	13.1	9.8	13.4	5.4
T ₂	48.4	8.8	12.1	10.3	12.2	5.3
T ₃	50.7	9.0	13.2	9.9	14.0	5.0
T ₄	45.7	10.2	14.0	10.1	15.8	5.5
T ₅	51.8	9.3	15.0	8.9	15.9	6.0
T ₆	58.3	9.7	18.1	9.2	20.8	5.3
<i>M. elongata</i>						
E ₁	75.2	8.6	22.4	8.3	31.3	4.2
E ₂	63.5	8.0	22.8	8.1	31.4	4.1
<i>M. mexicana</i>						
M ₁	56.7	9.4	18.9	10.4	18.2	5.8
<i>M. Laruei</i>						
L ₁	42.8	10.1	15.9	9.4	10.9	5.0

considered as a distinctive feature of the species.

Sexual reproduction.—*M. Laruei*, on the basis of its sex organs, appears to be most closely related to *M. mexicana* but differs from Shanor's species in the frequent basipetal type of development of the oogonia. Sex organs have been produced abundantly at all temperatures between 26° and 32°C. First a terminal antheridium is formed (fig. 42). Although it measures 8–19 μ long by 4–7 μ in width, this structure has the smallest average length, 11 μ , of any found in the isolates studied. From two to five antherozoids are produced.

The lateral outgrowth which soon forms beneath the antheridial cross wall may develop into a short branch bearing a terminal oogonium (fig. 45) or it may begin to expand to form a subantheridial oogonium (fig. 43, 46). This method of oogonial development corresponds closely to that of the hypogynous antheridia of *M. Taylora* and *M. elongata*. When the oogonium of *M. Laruei* is first cut off, the antheridium, now epigynous, lies in a direct line with the long axis of the hypha. Frequently, however, the oogonium rather than the antheridium assumes this alignment and the antheridium then appears lateral (fig. 44). Occasionally a second female organ may be cut off below the first. The oogonia are obpyriform or geniculate, depending upon whether they are terminal or intercalary. They measure 11–20 μ long by 7–12 μ in diameter at the widest point. Rarely two eggs are formed in an oogonium.

Oospores are produced in large quantities, but as yet their germination has not been observed. It is not known how long they will remain viable.

QUANTITATIVE DATA.—Quantitative data in regard to each of the species described above were obtained by taking measurements from camera lucida drawings. One hundred outline drawings were made of each of the reproductive organs, sporangia, oogonia, and antheridia, for each isolate.

The length and maximum diameter were recorded and the ratio of length to diameter calculated for each organ. The means of the lengths and diameters are shown in table 1 and the ratios of length to diameter are presented in graphic form in diagram 1.

These graphs show the ratio of length to diameter for sporangia, oogonia, and antheridia as these are found in the various species. In general, the sporangia are longer and more slender than the other organs, antheridia are intermediate, and oogonia are the shortest and proportionally the widest. The tendency toward shortness or elongation seems to be characteristic of all the organs of a given isolate. Where, for example, the sporangia are exceptionally long, as in isolate E₁ of *M. elongata*, the oogonia and antheridia are also long as compared to those of other isolates. Because the organs of *M. elongata* are not only longer than those of other species but are also narrower, the ratio of their length to diameter is especially conspicuous. Quantitative differences between the isolates of *M. Taylora*, *M. mexicana*, and *M. Laruei* are less pronounced.

DISCUSSION.—Of approximately sixty different soil samples examined in this investigation, ten have yielded fungi referable to the genus *Monoblepharella*. This would indicate that members of this genus are of not infrequent occurrence in the warmer regions of the Western Hemisphere. That no member of the genus was described until 1939 may be accounted for by their slow growth and consequent very late appearance in a gross culture. Thus it was sixty-eight years after Cornu (1871) published his discovery of species of *Monoblepharis* before Sparrow described *Monoblepharella Taylora*.

Although the similarities indicate a very close relationship between *Monoblepharella* and *Monoblepharis*, the differences are so extensive as to justify unquestionably Sparrow's separation of the

two genera. The motile zygote propelled by the persistent flagellum of the male gamete in *Monoblepharella* is a feature unique among known plants and, indeed, animals as well. The mycelium and reproductive organs of *Monoblepharella* are much more delicate than those of *Monoblepharis*. The sporangia are comparatively wider, and the zoospores frequently produced in more than one row in *Monoblepharella*; while in *Monoblepharis*, the sporangia, though much longer, are not much wider than their attendant hyphae and the zoospores are typically produced in a single row. In *Monoblepharella* sporangia are formed at temperatures ranging from 13°–36°C., and sex organs from 26°–32°C.; in *Monoblepharis* sporangia are formed predominantly at temperatures of 8°–11°C. and sex organs at about 21° C. (Sparrow, 1933). In *Monoblepharella* there is no oogonial papilla, the fertilizing antherozoid is not entirely taken into the oogonium, and the oospore is typically formed free in the water and is smooth walled; there is an oogonial papilla in *Monoblepharis*, the fertilizing antherozoid is entirely engulfed by the ooplasm, and the oospore remains in or at the mouth of the oogonium and in most species is covered with bullations. The refractive globules in the egg, zygote, and oospore of *Monoblepharella* are much larger than the globules in the corresponding cells of *Monoblepharis*.

And, lastly, there is a striking difference in habitat. Species of *Monopharis* have been collected on submerged vegetable debris in cool water; those of *Monoblepharella* have all thus far been recovered from tropical or semi-tropical soils.

Both Sparrow and Shanor have commented upon the similarity of *Monoblepharis regimens* Lagerheim and *Monoblepharis ovigera* Lagerheim to the known species of *Monoblepharella*. Although there is strong evidence that they should be placed in the same genus, it is felt that in order to avoid further confusion of the nomenclature no such transfer should be made until the sexual stages of these two imperfectly known species have been observed.

SUMMARY

A study was made of eleven isolates of *Monoblepharella* recovered from tropical or semi-tropical soils. A detailed description is given of the development and morphology of *M. Taylori*, followed by a brief statement of the outstanding characteristics and differences found in each of the other known species of *Monoblepharella*—*M. elongata*, *M. mexicana*, and *M. Laruei*. The genera *Monoblepharella* and *Monoblepharis* are compared.

DEPARTMENT OF BOTANY AND BACTERIOLOGY,
INDIANA UNIVERSITY,
BLOOMINGTON, INDIANA

LITERATURE CITED

- CORNU, M. 1871. Note sur deux genres nouveaux de la famille des *Saprolegniées*. Bull. Soc. Bot. France 18: 58–59.
- COTNER, F. B. 1930. Cytological study of the zoospores of *Blastocladia*. Bot. Gaz. 89: 295–309.
- COUCH, J. N. 1941. The structure and action of the cilia in some aquatic Phycomycetes. Amer. Jour. Bot. 28: 704–713.
- LAGERHEIM, G. 1900. Mykologische Studien. II. Untersuchungen über die Monoblepharideen. Bih. Kgl. Svensk. Vetensk.-Ak. Handl. 25, Afd. 3, No. 8, 1–42.
- LAIBACH, F. 1927. Zytologische Untersuchungen über die Monoblepharideen. Jahrb. Wiss. Bot. 66: 596–630.
- SHANOR, L. 1942. A new *Monoblepharella* from Mexico. Mycologia 34: 241–247.
- SPARROW, F. K., JR. 1933. The Monoblepharidales. Ann. Bot. 47: 517–542.
- . 1939. *Monoblepharis Taylori*, a remarkable soil fungus from Trinidad. Mycologia 31: 737–738.
- . 1940. Phycomycetes recovered from soil samples collected by W. R. Taylor on the Allan Hancock 1939 Expedition. Allan Hancock Pacific Expeditions, Publ. Univ. So. Calif. 3: 101–112.
- . 1943. Aquatic Phycomycetes (exclusive of the Saprolegniaceae and *Pythium*). University of Michigan Press. xix+785 pp.
- SPRINGER, M. E. 1945. Two new species of *Monoblepharella*. Mycologia 37: 205–216.

AUXIN IN LEAVES AND ITS INHIBITORY EFFECT ON BUD GROWTH IN GUAYULE¹

Paul F. Smith

IN A previous publication (Smith, 1944) the writer presented evidence to show that mature leaves of guayule (*Parthenium argentatum* Gray) may exert inhibitory influences on bud growth. This was noted in transplants as well as in undisturbed plants under certain conditions. In either case, latent axillary buds could be induced both to initiate growth in larger numbers and to start in less time when the leaves were removed or killed. It was suggested that this effect was under chemical control and that possibly auxin was the correlative substance, since it is known to serve this function in other plants (Went and Thiman, 1937; Ferman, 1938; Michener, 1942 and others). The purpose of the present paper is to report additional studies on this subject which demonstrate clearly that there is a relation between the transport of some agent(s) (apparently auxin) from the leaves to the stem and the retardation of growth of the lateral buds, and also that there is a correlation between the amount and distribution of the natural auxin and the growth of buds.

MATERIALS, METHODS AND RESULTS.—The plants used in the following studies varied somewhat in age and size in different experiments and will be described briefly in each case. In general, however, it may be said that the plants varied in age only from two to twelve months and were mostly outdoor nursery seedlings before being brought into the greenhouse for study. In two cases, the plants were young seedlings grown in flats of soil in the greenhouse.

The auxin determinations² were made according to the standard *Avena* method.

Girdling.—The inhibiting organs may be physiologically isolated by girdling either the petioles or the stem below the leaf junction (fig. 7-11). The parts distal to the girdle remain alive and functional for a considerable time. The girdling was accomplished by scalding with a stream of hot water directed at the desired point. After such treatment the buds proximal to the girdle develop at a rate comparable to those on defoliated plants (compare fig. 7 and 9). Usually the stem or petiole above the girdle thickens rapidly and becomes noticeably larger than the portion below the treated area. The anatomical changes accompanying this response have not been investigated.

Extraction of auxin.—Since there is as yet no standardized technique for extracting auxin from plant tissues, a workable method for guayule had to be found. The purified-ether extraction of fresh tissues (van Overbeek, 1938) was tried numerous

TABLE 1. Comparative auxin yields in degrees *Avena* curvature of samples dried at different temperatures. Extract from 3.0 g. dry leaf tissue taken up in 3 ml. of 1.5 per cent agar.

Temperature	Drying time (hours)	Mean curvature per gram dry tissue
35°C.	30.0	7.5
45°C.	12.0	8.4
55°C.	8.0	11.3
65°C.	3.5	14.3
75°C.	2.5	13.4
100°C.	1.0	7.4

times with many different samples of fresh leaves, stems and buds. Usually, however, no auxin could be obtained in this way as shown by the absence of curvatures in the *Avena* test. Attempts to diffuse auxin from leaves and buds also were unsuccessful. More than fifty leaves and nearly as many buds were used in the diffusion tests under both light and dark conditions, yet no curvature resulted when the agar blocks were tested.

Alkaline digests of fresh leaves in aqueous solution as used by Avery *et al.* (1942) and Haagen-Smit *et al.* (1942) on non-green material were tried on two occasions. No auxin was secured by this method.

When tissue samples, which had been dried and ground,³ were extracted with purified ether, auxin was consistently found. In order to determine the effect of the temperature of drying, a composite sample of leaves was harvested and portions were dried at six different temperatures until they were very low in moisture content (less than 0.5 per cent remaining). This was done by taking an equal weight of fresh leaves for each temperature and drying until each sample had reached the same dry weight. It was found that 99.5 per cent of the water was removed in a forced-draft oven at 100°C. in one hour. The other samples were dried until an equivalent amount of moisture was removed. The time for each temperature is shown in table 1.

Soxhlet extractions were made with three-gram samples and dilutions made of the extract so that the curvatures would not exceed 15°. Thirty-six *Avena* plants were used for each determination. The assays were repeated four times, each of which showed similar results to the one presented in table 1. Since the sample dried at the 65°C. temperature yielded more auxin than the others, that temperature was used in all subsequent tests.

³ It was found convenient to grind dried tissues through the 4 mm. screen of a Wiley mill. The samples used in the present study were all treated in this way.

¹ Received for publication December 11, 1944.

² The writer wishes to thank Drs. F. W. Went and J. Bonner, of California Institute of Technology, for their suggestions and help and for extending the use of their *Avena* laboratory, where part of the tests were conducted.

All ether extractions reported here were made by using soxhlet extractors. The time of extraction for maximum yield of auxin with dried and ground leaf samples was established by running several series with different times of extraction. In each case the purified ether was initially poured through the sample and the time of extraction considered to start at that time. The extraction flasks were heated before adding the ether so that boiling of the solvent started immediately and induced the flushing action of the extractors for the remaining period of extraction. Typical results from such an experiment are shown in table 2.

TABLE 2. *The relation of the time of extraction to the yield of auxin.*

Time	Mean curvature (deg. per gm. dry tissue)
15 min.	3.9
30 min.	7.4
45 min.	13.0
60 min.	12.5
180 min.	12.6
100 mgm. I.A. per L. = 11.8	

Three-gram samples of dried leaf tissue were used in each extraction and the extract was taken up in 3 ml. of agar. Further dilution of the extract was tried in subsequent tests with no relative increase in curvature. It may be seen that a maximum yield resulted in forty-five minutes, and the yield from these leaves is approximately equivalent to 110 micrograms of indoleacetic acid per kilogram of dried leaves. These leaves were from a different collection than those of table 1, yet the auxin yield is similar.

Ether extracts of guayule tissues yield relatively large quantities of substances other than auxin. Rubber, resin, essential oils, gums, waxes, chlorophyll and other plant substances are all ether-soluble and cause difficulty in taking up the extract in an aqueous agar solution. It was found advantageous, in some cases, to remove all of the fat-soluble components that would come out with petroleum ether, or benzene, before attempting ethyl ether extraction. Auxin-a is practically insoluble in these two solvents (Went and Thimann, 1937, p. 107). A comparison of the auxin yield of leaves from young plants of guayule (which were low in rubber, resins, etc.) with and without pre-extraction is shown in table 3. The pre-extraction time varied between six and fourteen hours depending on the material being extracted. The auxin extraction was for one hour. Different samples were used in the pre-extraction with each solvent so that although samples A and B are comparable, C is not. Since the auxin yield is apparently unchanged by pre-extraction with these solvents, this method was subsequently used in making determinations on woody samples which were rich in rubber and resin.

Distribution of natural auxin in guayule.—Using the above procedures, assays were run on normal eight-month-old seedlings that were hardened and dormant from winter conditions. These plants were harvested in April and fractionated arbitrarily into five tissue groups: (1) partially expanded leaves under three-fourths inch in length, (2) mature

TABLE 3. *Auxin yield of leaves of young seedlings of guayule with and without pre-extraction with petroleum ether or benzene.*

Sample	Mean curvature per gram		
	A	B	C
With petroleum ether.....	16.0	16.8	...
Without petroleum ether...	17.2	16.2	...
With benzene	11.5
Without benzene	11.2

leaves plus those partially mature and larger than (1), (3) terminal buds, (4) stems, and (5) the upper six inches of the tap roots. The results of a typical auxin determination are shown in table 4. From the data in table 4 it may be found that the leaves comprise slightly more than one-third (37.3 per cent) of the fresh weight of these plants yet they contain over two-thirds (71.3 per cent) of the auxin that was extracted. Not only do the leaves yield most of the auxin by this method of extraction but mature leaves yield considerably more than the immature ones. The apparent significance of this fact will be discussed later.

Molecular weight of guayule auxin.—Diffusion tests for determining the approximate molecular weight of the auxin obtained from guayule leaves were made following the general procedures of previous workers (Went, 1928; Heyn, 1935; and van Overbeek, 1940). The method is quite simple. Four agar blocks (0.88 mm. thickness) are stacked one on another in such a way that the upper one contains the plant extract. After a given length of time, at a known temperature, the blocks are separated and the auxin contents determined by the *Avena* test. Under these conditions, the distribution of the auxin in the four blocks is related to the size of the auxin molecule. By matching the values found for the four blocks with identical relative values in the diffusion table (Heyn, 1935) a corresponding diffusion coefficient (D) is found. This coefficient is related to the molecular weight as $M.W. = \frac{7 \times 1.07}{D_{22}}$. The results of three such tests are given in table 5. The average of the three tests gives a value of 166 which is close to that expected for indoleacetic acid. This strongly indicates that the auxin is either indoleacetic acid or a very similar compound, and not auxin-a or b. As a matter of fact a M.W. of 168 was obtained for indoleacetic acid from a parallel experiment to that used in determining the M.W. of the guayule auxin.

Auxin drop following defoliation.—If auxin from the leaves is the inhibiting agent on lower

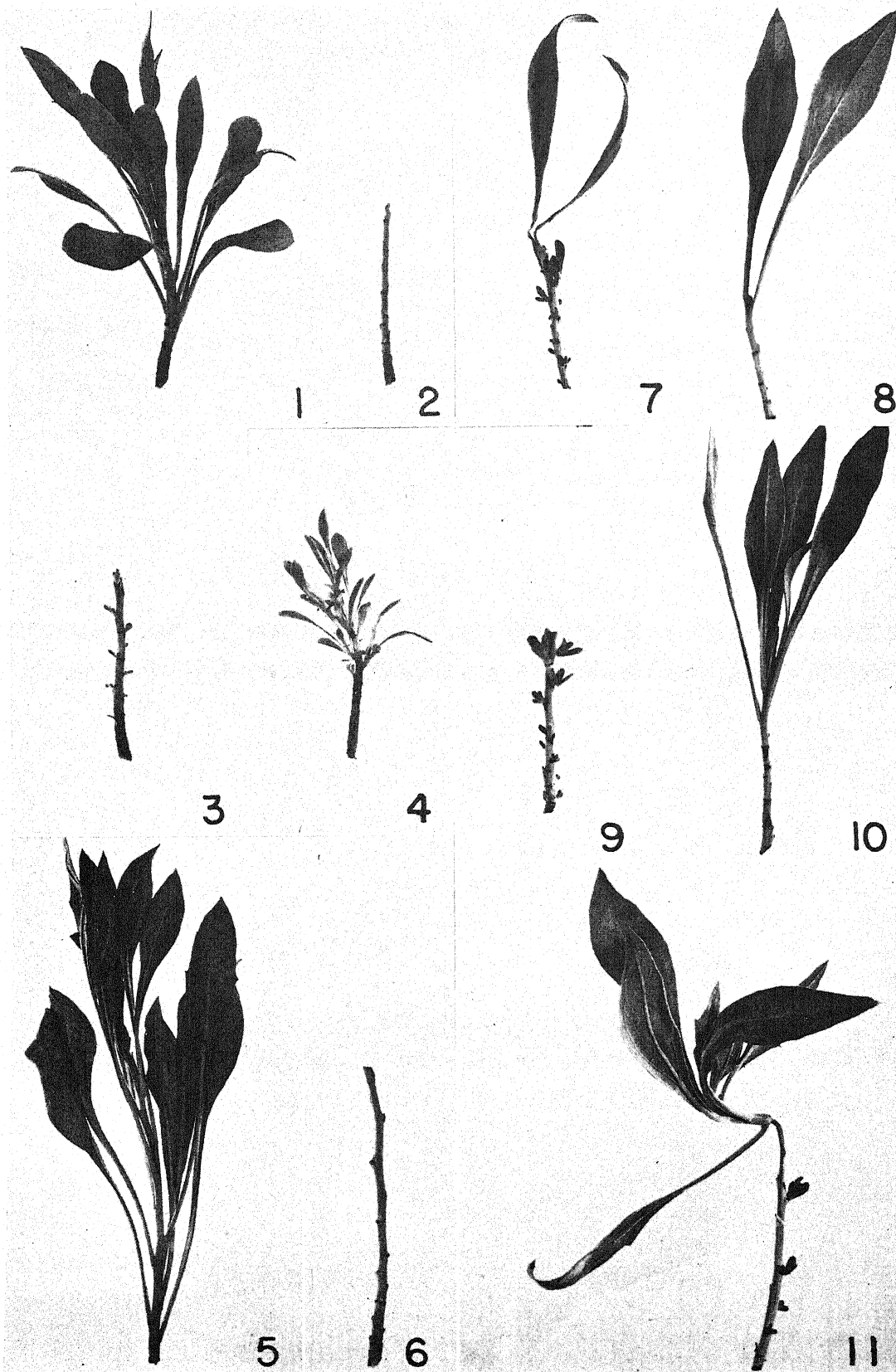


TABLE 4. *Distribution of the ether-extractable auxin in various portions of normal, 12-month-old guayule seedlings harvested in April, 1944. All extracts taken up in 1.0 ml. 1.5 per cent agar.*

Part of plant	Fresh wt. gms. per plant	Per cent H ₂ O in fresh tissue	Wt. of dry sample extracted in gms.	Mean curvature	Indoleacetic equivalents per kgm. fresh wt. (micrograms)	Per cent of total extracted auxin
Root (tap)	2.7	55.5	4	8.0	7.42	7.93
Stem	5.2	54.4	4	9.2	8.74	17.99
Terminal buds	0.5	65.0	2	9.6	14.00	2.77
Young leaves	2.2	68.0	1	9.7	25.87	22.53
Mature leaves	2.8	64.8	1	15.0	44.00	48.77
100 mgm. I.A. per L				12.0°		99.99

axillary buds, it is necessary to conclude that the auxin is transported through the stem to the buds. This in turn would suggest the hypothesis that stems of inhibited plants should be higher in auxin than stems of non-inhibited plants. In order to test this, groups of nursery plants were defoliated and transplanted into crocks; others were transplanted with the leaves on. After four days' time samples were harvested from both groups. The leaves were removed at this time from the second group, and assays for auxin were made on the dried stems. Table 6 shows the results of three such tests. Two types of seedlings were used in this experiment: (1) large seedlings that were about eight inches high and ten months old and (2) small ones that were about four inches high and five months old. Both groups showed visible bud growth seven days after defoliation.

TABLE 5. *Diffusion coefficients and molecular weights of three known auxins and the values determined for guayule auxin and 3-indoleacetic acid.*

	D ₂₂	Molecular weight	
Auxin-a ^a	0.414	328	
Auxin-b ^a	0.426	310	
3-indoleacetic acid ^a	0.567	175	
Guayule auxin	0.582	166	Mean = 166
Guayule auxin	0.570	173	
Guayule auxin	0.595	159	
3-indoleacetic acid	0.578	168	

^a Values given by van Overbeek (1940).

It may be seen in table 6 that the yields from the stems of large, freshly defoliated seedlings are

in close agreement with the yield shown in table 4 for stems of similar aged seedlings. These plants were from the same source and show that the auxin content of the stem changes little (in the presence of leaves) on standing four days after transplanting (compare stems in table 4 with freshly-defoliated stems of large seedlings in table 6).

TABLE 6. *Auxin assay on inhibited (freshly defoliated) and non-inhibited (4-day pre-defoliated) stems. Extract taken up in 1 ml. agar.*

Plant type	Dry weight of sample extracted gms.	Mean curvature	
		Freshly defoliated	Pre-defoliated
Large seedlings A	3.0	7.8°	4.5°
Large seedlings B	3.0	9.0°	4.8°
Small seedlings	3.0	17.1°	5.4°

Essentially the same response was obtained when young, established plants were defoliated without disturbing the root systems. The auxin assays of such an experiment are given in table 7, where it may be seen that the extractable auxin had decreased to one-third its original value after three days of defoliation. After six days several young buds on each plant had made sufficient growth to be visible and the auxin yield was slightly greater. Whether there is a significant difference between the auxin contents on the third and sixth days may be subject to doubt, yet the increase is consistent with a still greater increase at twelve days, at which time there were many leaves from one-half to three-fourths of an inch in length. This latter increase is one of 78.6 per cent over the amount

Fig. 1-11. Guayule seedlings used in certain auxin assays and girdling experiments.—Fig. 1-6. Typical seedlings used in auxin assays of the stems.—Fig. 1. The above ground portion of the plant at the start of the experiments.—Fig. 2. The bare stem three days after defoliation and decapitation with buds still invisible.—Fig. 3. A similar plant after six days. Note the many buds.—Fig. 4. A similar plant after twelve days with many young branches and leaves.—Fig. 5. Untreated plant after six days, which shows considerable growth as compared to the starting condition (fig. 1).—Fig. 6. A plant similar to that shown in figure 5, but with the leaves removed to show the absence of lateral bud growth.—Fig. 7. A twig with the terminal bud and all but two leaves removed and these with scalded petioles.—Fig. 8. A similar twig without scalding.—Fig. 9. A twig with all leaves removed. Note the similarity of the stems of figure 7 and 9 and the contrast of these two with figure 8 in respect to bud growth.—Fig. 10-11. Two twigs of the same general type, each of which had some of the lower leaves removed. The stem of the plant in figure 11 was girdled with boiling water. Note the growing buds on the plant in figure 11.

TABLE 7. *Auxin assays, in degrees curvature per Avena plant, of the stems of two-month-old guayule seedlings grown in soil flats in the greenhouse (July-August, 1944). The extract from 4.0 g. dry samples was taken up in 1 ml. agar.*

Treatment	Defoliated and sampled after	Defoliated at the start of the experiment and the stems sampled after the following lengths of time			
	6 days	0 days	3 days	6 days	12 days
Mean degree curvature	16.5°	16.3°	5.6°	7.0°	10.0°
General observations on lateral bud growth	No change (see fig. 5 and 6)	No change (fig. 1)	No change (fig. 2)	New buds appearing (fig. 3)	Many young leaves 1/2" to 3/4" in length (fig. 4)

present on the third day. In order to see if the auxin content of the stems was similar from day to day in the normal plant, a second harvest of freshly defoliated stems was made on the sixth day. The auxin yield was remarkably close to that found in the freshly defoliated stems of the initial harvest. Each determination was made from a mass sample of thirty plants per treatment and the curvatures are mean values of thirty-six *Avena* plants per sample. Typical stems of the various groups are shown in figures 1-6.

Of the four tests made on the decrease in auxin in stems following defoliation, it is striking that the auxin reached virtually the same level, even when the plants were of different ages and grown under different conditions. The change in auxin also was apparently independent of the disturbances of transplanting. This would logically be expected if auxin were acting in some direct way as the inhibitor of bud growth.

Inhibition by applied auxin.—Lanolin containing 3-indoleacetic acid was applied to both stem tips and petioles of defoliated plants. Mixtures containing 0.5 per cent, 2.5 per cent and 5 per cent indoleacetic were applied to petioles. Only the 0.5 per cent concentration was applied to stem tips. Sixteen plants were tested with each treatment. These were young plants varying from four to five inches in height. Thus, the "root crown" was at least four inches below the stem tip. Table 8 shows the number of plants with expressed lateral buds in fourteen days. The terminal buds were removed from all plants. It is to be noted that application of auxin directly to the stem was more effective than application to the petiole. Virtually complete inhibition was realized in this manner. Apparently auxin diffuses more slowly through petioles than through stem-tips. The fact that in some plants the buds several inches away from the stem tips started enlarging might indicate that auxin did not diffuse through the petioles at a rate adequate to get sufficient amounts through all the stem to cause inhibition. This might be explained in part on the basis of small contact areas in the petioles. It is perhaps of more importance, however, that the petioles usually turned yellow and in some cases died in the first few days. It is possible that the petioles were isolated by the formation of cork which has been

found to precede normal leaf fall (Addicott, 1945). In this experiment, the plants that developed buds near the crown did not develop buds on the upper

TABLE 8. *The effect of applied auxin on the expression of lateral buds after 14 days.*

Treatment	Number plants with buds growing	
	near stem tip	near root crown
Leaves on	0	0
Leaves off		
a. lanolin on stem tip.....	16	0
b. lanolin on petioles	15	0
c. 0.5% I.A. on stem tip.....	0	1
d. 0.5% I.A. on petioles.....	6	7
e. 2.5% I.A. on petioles.....	2	5
f. 5.0% I.A. on petioles.....	1	4

portion of the stem. Thus, for the total number of plants growing in each treatment, the values in the two columns should be added.

Strong inhibition of buds on defoliated stems of established young guayule plants has also been found when 1.5 per cent indoleacetic acid in lanolin is applied to decapitated stem tips.

Auxin and succulency.—Hardening of plants prior to transplanting is an old practice and is known to be beneficial for many types of plants. Guayule is no exception in this respect, a fact that has been known for some time and was recently substantiated by Kelley, Hunter and Hobbs (1945). It was considered possible that one of the differences responsible for the superior transplantability of hardened guayule plants might be a lower auxin content. To test this hypothesis, assays were made on the leaves, stems and tap roots of plants⁴ subjected to differential water application. The exact treatment, in respect to moisture, is presented elsewhere (Kelley *et al.*, 1945), and is only mentioned in general terms for the present purpose. The seedlings were all given abundant water until the plants were established, a period requiring about six weeks. Thereafter, plants subject to treatment I continued to receive an abundance of moisture, those given treatment III an intermediate amount, while those receiving treatment V received no fur-

TABLE 9. *The auxin content of plants grown under differential moisture treatments.*

	Leaves		Stems		Roots		Mean wt. per plant (dry)
	Per cent H ₂ O	Deg. curva- ture per gm. dry wt.	Per cent H ₂ O	Deg. curva- ture per gm. dry wt.	Per cent H ₂ O	Deg. curva- ture per gm. dry wt.	
Treatment I	82.8	33.2	82.6	16.3	76.0	9.7	1.19 g.
Treatment III	83.8	33.5	80.0	17.1	75.8	..	0.68 g.
Treatment V	72.8	16.6	67.1	10.0	62.1	4.3	0.34 g.

ther water application. Six weeks later (July 31, 1944) samples were taken. Plants subjected to treatments I were highly succulent, those receiving treatment III only slightly less so in appearance, whereas those given treatment V were quite small and non-succulent. The last-mentioned group was the only one in condition for easy transplanting. The auxin determinations are shown in table 9. One hundred plants of each treatment were massed and the tests for auxin made from aliquots.

The amount of auxin per unit of dry tissue was about twice as great in the two groups that received water during the whole growing period as in the hardened group. This response shows an inverse relationship between the transplantability of the plants and their auxin content at the time of transplanting. The hardened plants showed quicker starting and higher survival percentages than the two succulent groups (Kelley *et al.*, 1945).

DISCUSSION.—Any conclusions to be drawn from the present study are of course contingent on the validity of the extraction method used. No attempt was made to attain total auxin extraction. The auxin fraction given off in the first few hours of ether extraction from dried samples of guayule was found to be reproducible (table 2) and additional extractions were found to yield only traces of this substance. The auxin which is readily extracted in the first few hours is probably the important fraction available for physiological functions (Went, 1944) and the discussion herein presented is based on this assumption.

The general findings of the experimental work just presented seem to fit reasonably well into the picture of bud inhibition as developed from previous work. Certain phenomena, in the case of guayule, are different from those previously described for other plants. For example, leaves have been shown to cause inhibition of axillary buds in certain plants (Snow, 1929; Dostál, 1926, etc.) yet in no other plant has it been demonstrated that mature leaves may be the major source of the inhibitor (Smith, 1944). Leaves have been shown to produce auxin (Snow, 1929; Avery, 1935; Goodwin, 1937; Delisle, 1937, etc.) yet the production of this substance has consistently been found to be chiefly a function of young, partially expanded leaves. The present findings show that fully matured leaves of guayule yield more auxin on a unit of weight basis

than the partially developed leaves. Also, the terminal bud or coleoptilar apex of other plants has proved consistently to be a major source of auxin, yet in guayule the apical buds are scarcely any richer in auxin than the young stems.

The yield of auxin from guayule tissues is of the same order of magnitude as that reported for certain other plant materials. Yields of 0.5, 50, 50, and 80 micrograms per kg. fresh weight are reported for corn, pea, *Elodea*, and *Bryopsis* (van Overbeek, 1940); 24–31 micrograms for tomato leaves and stems (Gustafson, 1941); 0.02 and 110 micrograms for the scale leaves and stem tip of *Lilium longiflorum* (Stewart and Stuart, 1942), etc. The value of 44 micrograms found in mature guayule leaves seems to be about an average amount for vegetative plant structures. Much higher yields of auxin are known for certain materials. The grain of wheat, for example, has been found to yield large quantities of auxin upon hydrolysis (Haagen-Smit *et al.*, 1942) and Thimann, Skoog and Byer (1942) report large yields of auxin from *Lemna* upon enzymatic hydrolysis. These authors state that the large yields are due to the release of bound auxin by hydrolysis. This form of auxin is apparently not readily extracted by solvent extraction alone.

The experimental results presented leave little doubt that the leaves are important in the retardation of new bud growth after transplanting and also play a role in the branching of normal plants. The close parallel between the displayed inhibiting effect and the auxin distribution points strongly to auxin as the correlative substance. Just how auxin functions and what becomes of it seems to be as yet unknown for any plant. It is clear, however, that the auxin concentration is not a static entity but fluctuates with changed conditions. Light, temperature and water are known to change the auxin relations within the plant (Went and Thimann, 1937).

That auxin may be the inhibiting factor for lateral buds has been known for certain other plants. Went and Thimann (1937, pp. 211–213) state that this is the case with *Vicia* and *Pisum* and perhaps plants in general. The correlation between the amount of extractable auxin and inhibition of lateral buds in guayule is in complete agreement with the results of Michener (1942) who worked with potato tubers. In both cases the growth of buds followed a decrease or destruction of auxin.

* Plants kindly provided by Dr. O. J. Kelley, Soils Division, Guayule Research Proj. et.

The extraction of auxin from dried material has been a subject of some discussion in the literature. Ether extraction of dried samples has been previously used by other workers (Thimann and Skoog, 1940; Gustafson, 1941; Link *et al.*, 1941 and others), although smaller yields were obtained than with other techniques. Went (1944) found an increase in auxin yield as a result of drying. The present findings also show that extraction from dried guayule leaves yields many times as much auxin as from fresh leaves. In fact, virtually no auxin has been obtained from fresh leaves. It seems quite possible that reduced auxin destruction by drying is responsible for the increased yield (a view expressed by Went, 1944). The decrease in auxin yield by temperatures over 65°C. is perhaps due to auxin destruction by heat. Thimann *et al.* (1942) state that heating *Lemna* to 100° causes auxin destruction.

SUMMARY

Ether extraction of fresh tissues of guayule yielded little or no auxin. When the tissues were dried ether extractable auxin was consistently found.

The distribution of auxin in guayule differs from that previously described for other plants. The matured leaves yielded the greatest amount of auxin

on a unit of weight basis. The young leaves yielded less auxin, although they were much richer than the terminal buds, stems or roots. Succulent nursery plants showed approximately twice as much auxin on a dry weight basis as drought-hardened plants of the same age.

The growth of lateral buds was found to follow a decrease of auxin in the stems, in both transplants and plants with undisturbed root systems.

By determining the diffusion rate of the guayule auxin, a molecular weight of 166 was found, which indicates that 3-indoleacetic acid is the natural auxin.

Application of 3-indoleacetic acid in lanolin retarded the growth of axillary buds when applied directly to the decapitated stems. Only partial inhibition followed the application of auxin to the petioles; this appears to be due to quick abscission and poor transport through the petiole.

Steam girdling of both stems and petioles resulted in the immediate growth of proximally located lateral buds, which appears to indicate that the transport of auxin from leaf to bud was prevented.

BUREAU OF PLANT INDUSTRY,
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
SALINAS, CALIFORNIA

LITERATURE CITED

- ADDICOTT, F. T. 1945. The anatomy of leaf abscission and experimental defoliation in guayule. *Amer. Jour. Bot.* 32:250-256.
- EVERY, G. S., JR. 1935. Differential distribution of a phytohormone in the developing leaf of *Nicotiana*, and its relation to polarized growth. *Bull. Torrey Bot. Club* 62:313-330.
- , BERGER, J., AND B. SHALUCHA. 1942. Total auxin extraction from wheat. *Amer. Jour. Bot.* 29:612-616.
- DELISLE, A. F. 1937. The influence of auxin on secondary branching in two species of *Aster*. *Amer. Jour. Bot.* 24:159-167.
- DOSTÁL, R. 1926. Über die wachstumsregulierende Wirkung des Laubblattes. *Acta Soc. Sci. Nat. Moraviae* 3: 83-209.
- FERMAN, J. H. 1938. The role of auxin in the correlative inhibition of the development of lateral buds and shoots. *Rec. Trav. Bot. Neerl.* 35:177-287.
- GOODWIN, R. H. 1937. The role of auxin in leaf development in *Solidago* species. *Amer. Jour. Bot.* 24:43-51.
- GUSTAFSON, F. G. 1941. The extraction of growth hormones from plants. *Amer. Jour. Bot.* 28:947-951.
- HAAGEN-SMIT, A. J., W. D. LEACH, AND W. R. BERGREN. 1942. The estimation, isolation and identification of auxins in plant materials. *Amer. Jour. Bot.* 29: 500-506.
- HEYN, A. N. J. 1935. The chemical nature of some growth hormones as determined by the diffusion method. *Proc. Kon. Akad. Wetensch. Amsterdam* 38:1074-1081.
- KELLEY, O. J., A. S. HUNTER, AND C. H. HOBBS. 1945. The effect of moisture stress on nursery grown guayule with respect to the amount and type of growth responses on transplanting. *Jour. Amer. Soc. Agron.* 37. (In press.)
- LINK, G. K. K., V. EGGERS, AND J. E. MOULTON. 1941. Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs; its extraction with dry ether. *Bot. Gaz.* 102:590-601.
- MICHENER, H. D. 1942. Dormancy and apical dominance in potato tubers. *Amer. Jour. Bot.* 29:558-568.
- SMITH, P. F. 1944. Inhibition of growth in guayule as affected by topping and defoliation. *Amer. Jour. Bot.* 31:328-336.
- SNOW, R. 1929. The young leaf as the inhibiting organ. *New Phytol.* 28:345-358.
- STEWART, W. S., AND N. W. STUART. 1942. The distribution of auxins in bulbs of *Lilium longiflorum*. *Amer. Jour. Bot.* 29:529-532.
- THIMANN, K. V., AND F. SKOOG. 1940. The extraction of auxin from plant tissues. *Amer. Jour. Bot.* 27: 951-960.
- , F. SKOOG, AND A. C. BYER. 1942. The extraction of auxin from plant tissues. II. *Amer. Jour. Bot.* 29:598-606.
- VAN OVERBEEK, J. 1938. Auxin distribution in seedlings and its bearing on the problem of bud inhibition. *Bot. Gaz.* 100:133-166.
- . 1940. Auxin in marine algae. *Pl. Physiol.* 15: 291-299.
- WENT, F. W. 1928. Wuchsstoff und Wachstum. *Rec. Trav. Bot. Neerl.* 25:1-116.
- . 1944. Plant growth under controlled conditions. III. Correlation between various physiological processes and growth in the tomato plant. *Amer. Jour. Bot.* 31:597-618.
- , AND K. V. THIMANN. 1937. *Phytohormones*. New York.

OBSERVATIONS ON SPIRAL GRAIN IN TIMBER ¹

J. H. Priestley

OCCASIONALLY THE elongated elements of the wood in a tree lie rather uniformly at an angle to the long axis of the stem; this arrangement naturally affects the properties of the timber and is spoken of as spiral grain.² For some years observations have been made in these laboratories, as opportunity permitted, upon the grain in wood, and in particular study has been made (Misra, 1939) of spiral grain in softwoods (Conifers). The writer had gained the impression from these studies that a similar type of grain rarely occurred in hardwoods (Dicotyledons), though other types of grain seemed to have been incorrectly reported as spiral grain on several occasions. In 1942, however, the removal of iron railings in a street near the University of Leeds, England, drew attention to a trunk of elder (*Sambucus nigra* L.) that seemed to be a clear example of spiral grain. While this trunk was being intensively studied, other elder stems were found showing similar structure, and examples of spiral grain in other hardwood species were also found in and near Leeds. It transpires, however, that such spiral grain in hardwoods differs in fundamental points from the apparently similar phenomenon in softwoods, and a comparison of the two types of spiral grain is therefore attempted in the following pages. While it is naturally impossible to make broad generalizations from the comparatively few specimens studied here, certain tentative suggestions can be made as to the factors associated during growth with the ultimate production of spiral grain.

It seems desirable to preface the present observations upon spiral grain in hardwoods, a subject that has so far received little attention, with a brief statement of the characteristics of spiral, or twisted, grain in softwoods. Examples of spiral grain in softwoods have frequently been recorded and studied, but the observations require re-examination after considering the apparently related phenomena in the hardwoods.

SPIRAL GRAIN IN THE SOFTWOOD.—A very full account of this type of wood, as it occurs in the Indian chir pine (*Pinus longifolia* Roxburgh) is given by Champion (1925) and the literature prior to that date is fully cited. Our observations, in particular many made by Misra (1939), first in this country and then in India on the same species, support in the main the generalizations of Champion. We are in complete agreement that the wood of the

first year shoot is invariably straight, that the twisted grain may appear in the wood of the second year or later, and that it is usually left-handed (in the sense in which this term is used of the cutting spiral on screws, a left-hand twist seen on the outer surface of the wood will run from the left at the top to the right below) when it first appears.

In a young tree the twist will usually become more marked (i.e., the angle that the wood elements make with the vertical will become larger) as the trunk grows older; the twist will therefore at first be more marked at the base of the sapling, especially in the hypocotyledonary region, and will diminish upward until it is entirely missing in the first year extension shoot. The spiral is never continued far down into the root.

Champion describes the branches from a trunk with spiral grain as showing "a tendency to be more intensely twisted" (1925, loc. cit. p. 17). Our observations on softwoods grown in England led us at first to challenge this statement. In English-grown softwoods striking cases of twisted grain are not of such frequent occurrence as in the chir pine, but in all cases examined, horizontal branches, even on trunks with twisted grain, showed relatively straight grain. Misra confirmed this conclusion after his return to India on young trees of the chir pine and we found ourselves in complete agreement with Jacobs (not dated) who reported that, in *Pinus radiata* grown in Australia, twisted grain was only found in branches which were growing as substitute leaders or in competition with the leader. Attention is drawn by Canning (1915), however, to the "contorted" forms of branches frequently seen in overmature trees of chir pine. These trees often show a right-handed twist on the trunk, and in the "contorted" branches the degree of twist is often still greater. In England, old trees of *Pinus sylvestris* often have a number of branches growing irregularly upwards, and on these also we have now noted a late-developing right twist.

After a few or many years of increasing inclination of the grain in the trunk, trees of *Pinus longifolia* with left-handed spiral grain, as they approach maturity often show a gradually diminishing spiral inclination, and in mature, or overmature, timber the wood elements in the outer layers of wood may run vertically or a reversal of the direction of twist may be noticed. In this species a right-handed twist is described as appearing relatively late in the life of the tree, possibly after 150 years, but in *P. sylvestris* and *P. montana* it is reported in trees with a diameter of about 6 inches, and in *Picea excelsa* it appears as early as the 10th year (Champion, 1925).

SPIRAL GRAIN IN THE HARDWOOD.—Nearly all discussions of spiral grain, though they may refer incidentally to hardwoods, are based, in any analysis

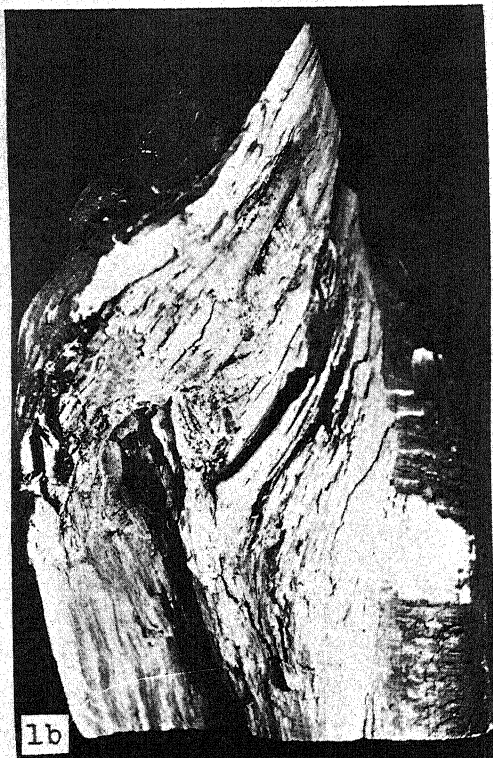
¹ Received for publication July 25, 1944. The editors regret to report the death of Professor Priestley on October 31, 1944.

The author desires to express his thanks to Mr. A. Millard for all photographs and for his diligent and successful search for spiral-grained hardwoods, and to Miss K. M. Mattinson for preparation of the manuscript for press.

² The term "spiral" is retained since it is already in common usage, though technically "helical" would be more correct.



1a



1b



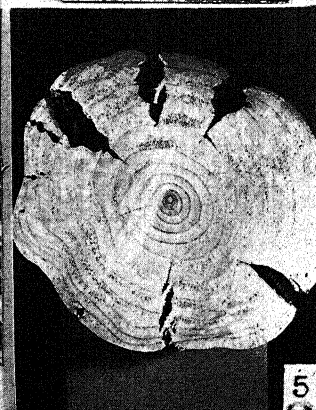
2a



3b



4



5

of detail, on softwood examples and it is assumed that the phenomenon will be of the same general character in hardwood species. This assumption we shall find to be invalid, and difficulty arises from this cause at the outset, since a limited examination, such as would justify a report of spiral grain in a softwood, does not suffice in the case of a hardwood. If the bark is removed from a localized area of a softwood trunk and the wood elements show a definite tilt, then, apart from branch entry disturbances, spiral grain may safely be assumed; in a hardwood, tilted elements in a localized area free from branch insertions provide no safe criterion of the occurrence of spiral grain. In many hardwoods, especially in older trees, the course of the wood elements may diverge widely from the vertical but in so variable a manner that the grain is better described as irregular or wavy. In wavy grain, seen fairly frequently in ash and oak, the same undulating course may be followed throughout the older rings over many years, so that the wood, if split, shows on the radial face long transverse ridges alternating with grooves (Desch, 1938 loc. cit., fig. 59), though any ridge or groove followed outwards may gradually fork into two as the waviness becomes more accentuated. In these cases it seems clear that the course of the files of cambium elements has slowly become and remained undulating, and the same general wave is repeated in each successive increment of wood. This is quite a frequent phenomenon. More frequent still, perhaps, especially in Indian and tropical timbers generally, is a combination of such an undulating course of the wood elements with rapid and repeated changes in direction of the elements in successive radial increments (Desch, 1938, fig. 58). This interlocked grain has been fully described by Martley (1922) under the term double cross grain.³ It is also seen in English timbers such as the elm (*Ulmus campestris* L.), and is responsible for much exasperation when billets are being split for the fire.

Some original reports of spiral grain in hardwoods have turned out to be cases of either wavy or interlocked grain, but the trunk of elder now available for examination showed (fig. 1a) a strongly marked, right-handed spiral grain. This elder is

³ Interlocking grain is described in British Standards (1938) as the crossing of alternating "spiral grain." No such case has been seen by the writer and in view of the facts brought to light about spiral grain in hardwoods in the following pages this definition seems to need reconsideration.

Fig. 1-5.—Fig. 1. (a) Part of main stem of *Sambucus* showing spiral grain. Cambium still active under strip where bark is retained. (b) Part of trunk split longitudinally; the pith channels show that the main stem ends on left, and that the axis is continued sympodially by growth of branch on right. Note how spiral grain on right is obviously running smoothly from branch to main axis.—Fig. 2. A piece of *Sambucus* stem with twisted grain has broken into two twisted wood fragments, through the pith. (a) Shows the two pieces fitted together. (b) In the upper fragment the pith is seen as a dark groove, in the lower, tissue still fills the lower part of it. The increasing tilt of the grain with increase in diameter is indicated.—Fig. 3. Part of stem of *Clematis*, showing (a) left-handed twist in sclerenchyma fibers, (b) fibers removed from same stem showing a deep crack in wood which has about the same direction.—Fig. 4. *Castanea*, bark on main trunk showing outermost flakes running nearly vertically over left-sloping deeper cracks.—Fig. 5. Cross sectional view of block from twisted larch, showing cracks after drying for 3 years. Many of the rings are markedly excentric.

unusual in having a single main trunk, and in its length of 2.5 meters it included three complete turns of the spiral. Above this level the trunk broke down into a number of branches, of which the two main branches continued to show a spiral grain, while three others were straight-grained; at higher levels most of the branches became moribund, and the main spirally-twisted branches terminated in broken stumps, while the only living shoots were borne on irregularly branched systems in which the wood was straight-grained.

The inclination of the spiral is very similar on the trunk and on the bases of the twisted branches and, as the internal structure of the trunk was examined, it became evident, as might be expected in the elder, that the trunk, although appearing as one stem with spiral grain at about the same inclination throughout, was actually of sympodial construction. Figure 1b shows one region of the trunk split longitudinally, about one meter above the ground line, where on the right is seen the pith of a branch which continues the direction of the main axis while the previous main shoot ends as a dead stump on the left. On the right of this section of the trunk there is a clear indication from the plane of splitting that the twisted grain runs at about the same angle both on the original main axis below and on the branch above. The tree had suffered hard usage and much of the bark had been stripped from areas exposing dead wood, the only strip of living bark followed a spiral course which conformed generally with the inclination of the spiral grain in the underlying wood. Evidently, as might be expected, the long axis of the fusiform initials of the cambium lay at the same inclination as the wood within.

This clear case of spiral grain in elder is distinct from wavy grain because the wood elements lie on the whole parallel with one another but at an angle to the long axis of the stem. It differs from cross or interlocked grain because the inclination of the spiral does not change in direction from one layer of wood to another. As a result, when the trunk is cut into sections and the wood dries, cracks appear along radial lines from the periphery to the center and the wood finally splits into segments with obliquely tilted radial faces (fig. 2).

The difference from spiral grain in the softwood is equally fundamental. In the softwood the wood elements of the extension shoot of the first year seem without exception to run vertically, while in

the elder specimen it is equally clear that the oblique course of the wood already prevailed in the first year shoot and that the spiral twist then imparted to the vascular strands has been maintained and has determined the orientation of the wood elements in every subsequent year. The result is a progressively increasing tilt to the grain of the wood resulting from the purely geometric condi-

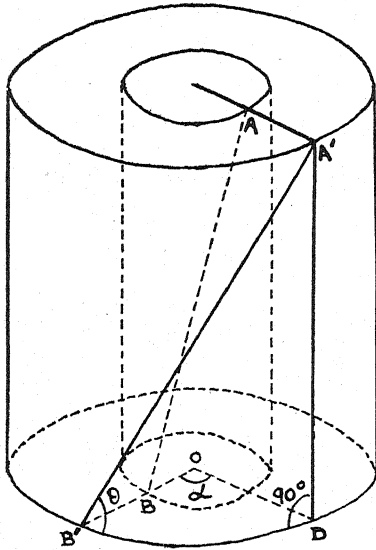


Fig. 6. Diagram showing how, if the pitch is determined in the first year wood, at AB, it will increase with increasing diameter, as at A'B'.

tions due to increase in girth. Thus in figure 6 it will be seen that if the tilt in the wood is represented by a line joining rays A and B then, if the rays run a straight radial course outwards, with increasing girth of the stem the tilt will increase on the surface of the wood, since the cambium cells remain in the same relative position in the growing cambium layer.

In two pieces of elder stem where radial cracks permitted the observation of the slope of the wood, the angles made with the horizontal were measured with a protractor at different distances outwards through the thickness of the wood. The figures are given in table 1, and the cotangents of the angles are plotted against distances from the pith in figure 7. In the branch these cotangents show a linear relation to girth,⁴ so that the decreasing angle to the horizontal with increase in diameter is determined purely by the geometry of the system. In the older stem the angle could not be determined in the first year wood since both pith and inner wood were rotten. The radial distances through the wood were

⁴ This is to be expected from the geometry of the spiral, for in any section of the trunk as shown in figure 6, we have the relations:

$$B'D/A'D = \cot \theta$$

$$B'D/B'O = a$$

$$\text{Hence } B'O = (A'D/a) \cdot \cot \theta$$

Since $A'D/a$ is constant, then $\cot \theta$ is proportional to the radius $B'O$.

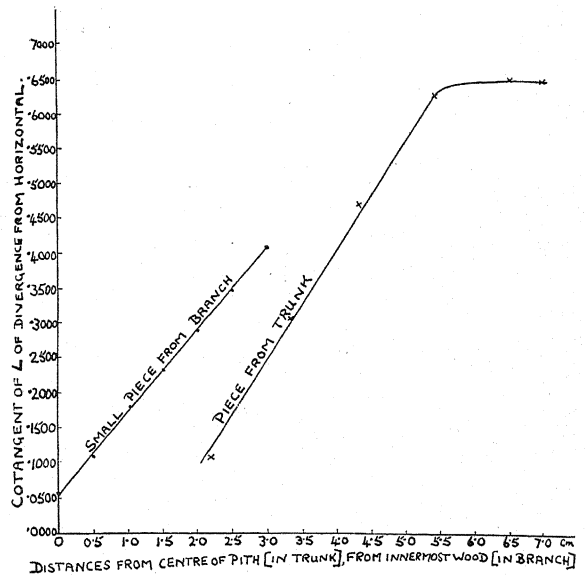


Fig. 7. Graph showing cotangent of angle of divergence of grain from the horizontal plotted against distance from center in trunk and branch of *Sambucus*.

therefore measured from a central point in the stem. Here again the same relationship holds, as is clear from figure 7, until the outermost layers of wood are reached. The annual increments of wood were particularly narrow in the outer rings in the sector of the trunk in which this radial crack was present and consequently the rays in these outer rings, instead of continuing to diverge, remained almost parallel. Thus the inclination of the twist, determined by the torsion of the original one-year shoot, was modified progressively so long as each new increment of wood had the normal increasing periphery, but remained constant when the increment of wood was reduced in this localized region

TABLE 1. *Sambucus*—Inclination of the wood grain to the horizontal.

(1). Piece from trunk.		
Distance from center		Inclination
7.0 cm.		57°
6.5 cm.		57°
5.4 cm.		58°
4.3 cm.		65°
3.3 cm.		73°
2.2 cm.		84°
(2). Small piece from branch.		
Distance from innermost layer of wood		Inclination
3.0 cm.		68°
2.5 cm.		71°
2.0 cm.		74°
1.5 cm.		77°
1.0 cm.		80°
0.5 cm.		84°
0.0 cm.		87°

so as to lead to no further divergence of the rays.

As a further illustration of this geometric relation, it may be mentioned that an angle of 3° (the inclination to the vertical on the innermost ring of the branch in table 1 (2)) will subtend a base line of 2.5 cm. (the periphery of the innermost ring of wood) at a distance of some 48 cm.; in this distance therefore wood elements inclined at the angle of 3° will have just completed one circuit of the periphery. In this branch the spiral shown on the surface layer of wood made a complete turn in about 47 cm., so that the turns of the spiral in the wood in the center of the stem and in the outermost wood were completed in the same longitudinal distance. From table 1 (2) it will be seen that the outer layer of wood on the 3 cm. radius made an angle of 22° with the vertical. At a distance of 48 cm. this angle should subtend a base line of some 20 cm.; the circumference of the wood of the branch when measured proved to be about 22 cm.—quite a good agreement when allowance is made for the small variations in the angular inclination of the wood in this layer.

Thus in the elder the spiral grain of the wood has been determined throughout the whole radial depths of the wood by a spiral twist initiated during the extension growth of the first year shoot. The original torsion, thus seen to determine the production of spiral grain, would arise as a natural consequence of any unequal extension of the growth units built around the leaf trace systems in this decussate shoot (Priestley, 1929; Griffiths and Malins, 1930), which would cause the original trace systems to lie at a slight angle in the axis. This torsion may be visible in the course of any ridges or grooves on the surface of the internode; though such effects have not been noted in any first year shoots of *Sambucus*, they are relatively common in other Dicotyledonous shoots with elongated internodes. Good examples are often provided by the elongated internodes of *Clematis*; as the stem increases in girth the epidermis ruptures, leaving exposed on the surface the sclerenchyma strands; when the fibers are removed and the wood surface is exposed, a spiral crack is seen in the wood showing that the grain in the secondary wood has the same spiral twist as the phloem fiber strands (fig. 3). Evidently the fusiform elements of the cambium in the slightly twisted trace strands have given rise throughout their activity to wood elements which are also lying in a spiral around the stem.

That no similar evidence of torsion was ever noticed on the first year shoots of *Sambucus* is perhaps not surprising. The markedly twisted branch used to obtain the data given in table 1 had an inclination of only 3° from the vertical in its first year shoot, and torsions of this order will not easily be recognized. This is even truer of other examples studied, e.g., lilac (*Syringa vulgaris* L.) in which the initial inclination of the strands in the first year twig must have been even less than 3° .

A still more striking case was observed in specimens of *Castanea sativa* Mill., the Spanish chestnut, in which well-marked twist is a common feature. A beautiful specimen is figured by Elwes and Henry (1909, plate 237) of a Japanese chestnut (*C. crenata* Sieb. and Zucc.) from the Atera Valley. Excellent twisted specimens of *C. sativa* were found in the Deer Park near Otley, three with a well-developed right-handed twist and three with a left-handed twist. One large fallen trunk showed a well-marked right-handed twist throughout the length available (some 40 feet). A cross section taken from the middle of this trunk, which was about 0.7 meter in diameter, showed the wood on the surface lying at an angle varying between 15° and 20° from the vertical. Farther in, this twist gradually diminished. It seems clear that the grain is uniform throughout the trunk (though the occasional "ring shake" almost invariably present in Spanish chestnut grown in this area must be remembered) and that it gradually diminishes towards the center to a practically undetectable slope.

Our point may be illustrated by a photograph of the bark of a fine left-twisted trunk of *Castanea*. In this tree also the divergence from the vertical in the wood of the slender sapling must have been negligible, and at that time the strands of fibers and wide rays in the phloem, the course of which determines the position of the original cracks in the widening sheet of bark, must have been practically vertical. As the axis increases in girth, the increasing divergence from the vertical shown by the wood must be shared by the inner deposits of phloem fibers running between the rays and thus this increased slope will show in the younger but deeper cracks in the bark. In many places on this trunk (fig. 4), outside the deep cracks which reveal the twist in the later layers of bark, can be seen slender, superficial strands of bark, the remains of those first laid down, the direction of which on the tree is practically vertical. These vertical strips have to bridge the sloping cracks farther in and ultimately, of course, must be torn off by the widening of the cracks below.

As a consequence of these observations we are faced with the necessity of a new attitude towards spiral grain. It still remains true that twisted grain is relatively rarely reported for hardwood trees, and in view of the slight tilt in the trace system of the original extension shoot necessary to produce this effect, the remarkable fact seems to be, not its fairly frequent occurrence in *Castanea* and its occasional appearance in *Sambucus*, *Syringa*, etc., but rather the prevalence of straight grain in the majority of Dicotyledonous timbers.

It is perhaps worth noting that most examples, both of torsion in the internodes and of spiral grain, seem to be reported from decussate plants where only two pairs of trace systems are usually present in one internode, and where the torsions must be due to unequal development of the two traces of one pair which lie relatively isolated in the inter-

node. *Castanea sativa* has a variable phyllotaxis which is usually $\frac{2}{5}$ in the main vertical shoots, but $\frac{1}{2}$ in the horizontal shoots. In *Sambucus*, *Syringa* and *Castanea* the main axis is sympodially constructed, and the occurrence of torsions in the first year extension shoots seems very generally associated with the rapid curvature of a lateral shoot as it assumes the vertical position. If in *Castanea* this curvature occurred in a shoot with $\frac{1}{2}$ phyllotaxis, this might facilitate the production of torsion, and the $\frac{1}{2}$ phyllotaxis on horizontal branches might also be associated with the marked persistence and even intensification of twist in some of the horizontal branches of this tree.

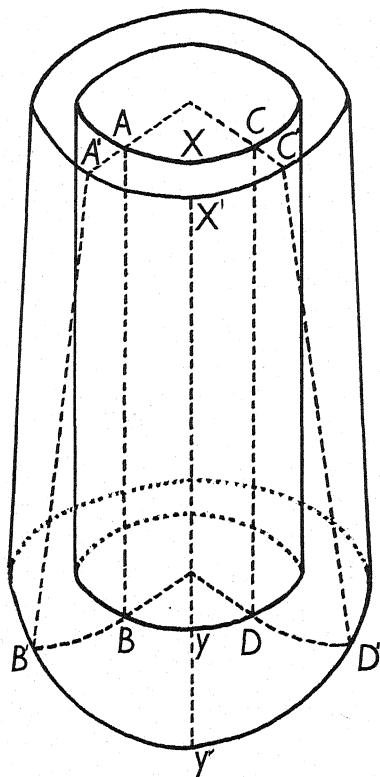


Fig. 8. Diagram showing effect of a basal excentricity in producing oppositely sloping grain on the two flanks of the "buttress."

If the development of torsion and thus of twisted grain in the Dicotyledon is to be associated with elongated internodes and a trace system in which differentiation may not be well synchronized because the traces in the same internode are relatively widely dispersed, then such torsion and twisted grain should not be expected in most Conifers where the phyllotaxis system has usually a number of orthostichies and the internodes are comparatively short. Furthermore in the softwood the original spiral is not indifferently right-handed or left-handed, as appears to be true for the hardwood, but is fairly constantly left-handed in direction, and while this left-handed twist is developed it is usually not evident on horizontal branches.

A brief mention is necessary of a basal twist—very frequent in the bole of hardwoods—which is not continued upwards in the trunk as a continuous spiral. A case in ash (*Fraxinus excelsior* L.) was examined, and it is evident that it may be traced to the localized expansion of girth of the trunk at the base where it is buttressed over the main supporting roots. Any localized excentricity in girth is liable to affect the course of the grain, as may be judged by the fact that the rays no longer take a straight radial course through the wood but diverge more widely where the greater growth is proceeding. An attempt is made in figure 8 to indicate the effect of a single basal excentricity in producing inclination in an otherwise straight-grained tree. At the base level, the divergent growth will convert the original straight grain at AB and CD on the more slender trunk into the respectively left-inclined A'B' and right-inclined C'D' on the older trunk, while around the plane X'Y' the wood should remain straight-grained.

SPIRAL GRAIN IN THE SOFTWOOD RE-EXAMINED.—As we now turn to reconsider spiral grain in softwoods, we are confronted with a condition which cannot be associated with an original torsion in the extension shoot, but is an effect developed during a later phase of cambial activity. All previous discussions of spiral grain (although this may not be expressly stated by the writers) have been based upon the more frequently observed facts in the softwood and throughout all these discussions there is no recognition of the possible effect of the geometry of the system as a factor likely to flatten the spiral as girth increases. The reason for this is obvious; the inclination is not determined in the first year shoot in the softwood, so that the idea has never been present in the mind of the investigator that a turn of the spiral, being thus pre-determined, should always continue to be completed in the same length of axis, as girth increases. In the softwood, after the first year the slope varies gradually from year to year, but each varying slope may correspond to a spiral that completes a circuit of the axis in a different length. This is apparent if the angle with the horizontal at different radial depths in a twisted softwood is determined and the cotangents of the angles⁵ are plotted against radial distance from the center. Such figures are given in table 2 and are plotted in figure 9 for various specimens of *Pinus radiata*, supplied to us by Dr. M. R. Jacobs from Australia. The cotangents show no sign of the strict proportionality with radial distance shown by the hardwood; they may increase rapidly at first as the left-handed spiral flattens rapidly, then their increase becomes very slow until they fall off to nil (or negative values) as the grain again becomes straight or finally even becomes right-handed.

The same point appears very clearly if the splitting of a twisted softwood log is studied as the wood dries and shrinks. Figure 5 is photographed

⁵ See footnote 4 on p. 270.

from a twisted European larch, supplied by the Forestry Commission, which has been drying in the laboratory for more than three years. Fifteen rings of growth are represented in this block. No crack opening to the surface has spread inwards farther than the eighth year and some cracks, though well open in the wood, do not reach the surface. Certain details in these cracks are most suggestive, but one point which immediately emerges is that they are present in rings which are markedly excentric. All cracks are very different in character from the clear, radial cracks in the hardwood with its uniformly coiled spiral grain (fig. 2).

Already in the hardwood we had cause to see that the slope of the grain might be affected in later years by excentric radial increments. In the softwood the influence of torsion in the first year shoot is excluded, but excentricity of radial growth in subsequent years may affect the direction of the grain. It is suggestive in the first place, that apart from an observation by Braun on *Pinus sylvestris*

by Misra's observations, is that this excentricity itself is distributed around the stem in a spiral. This observation had previously been made on extreme cases by Champion (1925, loc. cit. fig. 2 and p. 6).

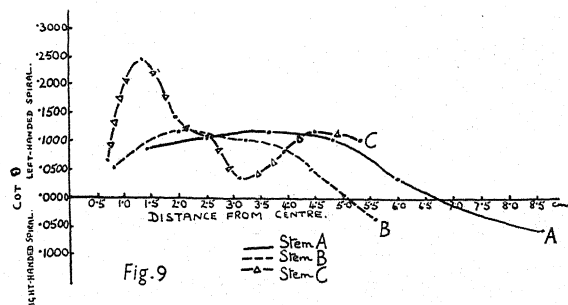


Fig. 9. Graph showing the cotangent of the angle of divergence of the grain from the horizontal plotted against the distance from the center in *Pinus radiata*.

TABLE 2. *Pinus radiata*. Angles of inclination of wood grain from the horizontal.

Distance from center	Angle (θ)	Cot θ
1. Stem A		
1.4 cm.	85° (left-handed)	0.0875
2.5 cm.	84 (left-handed)	0.1051
3.6 cm.	83 (left-handed)	0.1228
4.8 cm.	84 (left-handed)	0.1051
6.0 cm.	88 (left-handed)	0.0349
8.6 cm.	87 (right-handed)	0.0524
2. Stem B		
0.8 cm.	87° (left-handed)	0.0524
2.0 cm.	83 (left-handed)	0.1228
3.2 cm.	84 (left-handed)	0.1051
4.4 cm.	87 (left-handed)	0.0524
5.6 cm.	88 (right-handed)	0.0349
3. Stem C		
0.7 cm.	86° (left-handed)	0.0699
1.3 cm.	76 (left-handed)	0.2493
1.9 cm.	82 (left-handed)	0.1405
2.5 cm.	84 (left-handed)	0.1051
3.1 cm.	88 (left-handed)	0.0349
3.9 cm.	85 (left-handed)	0.0875
4.5 cm.	83 (left-handed)	0.1228
5.3 cm.	84 (left-handed)	0.1051

(1854, loc. cit. p. 449), it is generally agreed that the first year softwood shoot shows no recognizable twist. Champion and Misra both studied this matter closely and while Misra sometimes noted very slight excentricity, he never observed twisted grain in the first year shoot; in later years excentricity, often associated with spiral grain, frequently becomes prominent.

Excentricity, however, would not produce spiral grain if it ran as a continuous vertical inequality of increment, and the reason that excentricity produces spiral grain, as established for the first time

The degree of excentricity and the position of maximum excentricity in the softwood trunk vary in successive annual increments, and it is not surprising that the actual "spiral" followed by the wood elements varies from year to year. In fact the wood elements do not follow a spiral course in the same definite way that they do in the examples of spiral grain previously described in hardwoods. In the softwood, radial files of elements, which lie beneath one another and with their ends interlocked, have received a slight tilt due to the effect of excentric radial growth upon the cambium initials from which they are formed. In view of the contrast which obviously exists between so-called "spiral grain" in softwoods and hardwoods, one feels that it might help towards a better understanding of the phenomena if "tilted grain" were used as the term in softwoods to distinguish it from "spiral grain" in hardwoods.

SUMMARY

As a result of an opportunity to study certain cases of spiral grain in hardwood trees (*Sambucus nigra*, *Syringa vulgaris*, *Castanea sativa*), fundamental differences are found between the structural features in hardwoods and softwoods. In hardwoods, spiral grain is the result of a twist given to the trace system and cambium cylinder in the primary axis; the spiral inclination thus given to all secondary vascular elements persists throughout radial growth and is therefore accentuated each year by the geometrical results of expanding the spiral with the increasing girth of the woody axis, though the files of wood elements still complete the circuit of the axis in the same longitudinal distance.

By contrast, in the softwood the so-called spiral course of the wood elements is really fictitious. In the first year wood the grain is always straight, the inclined course of the wood elements arises during the second or later years; it is due to a tilt given to

the cambium initials, which results in the originally vertical elements in the radial files becoming tilted but not otherwise altering their position. There is no sense in which any file of elements, traced longitudinally, completes a circuit of the axis; and the gradual change in tilt of the elements, which occurs in later years, has no direct connection with the increasing girth of the axis. On the other hand a close correlation exists between the occurrence of the so-called spiral grain in the softwood and the occurrence of excentricity in the radial increment. The position of maximum thickness in any excentric cylinder of wood added to an upright softwood axis has recently been shown to follow a spiral course along the length of the axis and it is shown that this phenomenon adequately accounts for the development of a tilt in the fusiform initials of the cam-

bium. If the position of maximum thickness follows a left-handed spiral course in the axis, the tilt imparted to the wood elements gives the appearance of left-handed spiral grain; if the position of maximum thickness follows a right-handed course the tilt to the wood elements suggests a right-handed spiral grain.

In order to assist in a fuller realization of the fundamental differences, it is suggested that it would be helpful to restrict the term "spiral grain" to the phenomenon in hardwoods and describe the grain now called spiral in the softwoods as "tilted grain."

DEPARTMENT OF BOTANY,
LEEDS UNIVERSITY,
LEEDS, ENGLAND

LITERATURE CITED

- BRAUN, A. 1854. Über den schiefen Verlauf der Holzfaser und die dadurch bedingte Drehung der Bäume. Bericht ii. die z. Bekanntmachung geeigneten Verh. der. Königl. Preuss. Akad. der Wiss. Berlin 432-484.
- BRITISH STANDARD TERMS AND DEFINITIONS APPLICABLE TO HARDWOODS AND SOFTWOODS, No. 565, London. 1938.
- CANNING, F. 1915. Twisted fibers in chir pine. Indian Forester 41:112-116.
- CHAMPION, H. G. 1925. Contributions towards a knowledge of twisted fiber in trees. Indian Forest Records 11, Part 2:1-70.
- DESCH, H. E. 1938. Timber, its structure and properties. London.
- DE VRIES, Hugo. 1892. Monographie der Zwangsdrehungen. Pringsheim's Jahrb. für Wiss. Bot. 23:13. (Opera e Periodicis collata 5:232-406.)
- ELWES, H. J., AND AUGUSTINE HENRY. 1909. The trees of Great Britain and Ireland 4. Edinburgh.
- GRIFFITHS, ANNIE M., AND MARJORIE E. MALINS. 1930. The unit of shoot growth in dicotyledons. Proc. Leeds Phil. Soc. (Scientific Section) 2:125-139.
- JACOBS, M. R. (not dated). The occurrence and importance of spiral grain in *Pinus radiata* in the Federal Capital Territory. Leaflet No. 50, Commonwealth Forestry Bureau.
- MARTLEY, J. F. 1922. Double cross-grain. Ann. of App. Biol. 7:224-268.
- MISRA, P. 1939. Observations on spiral grain in the wood of *Pinus longifolia* Roxb. Forestry 13:118-133.
- . 1943. Correlation between excentricity and spiral grain in the wood of *Pinus longifolia*. Forestry 17:67-80.
- PRIESTLEY, J. H. 1929. Cell growth and cell division in the shoot of the flowering plant. New Phytol. 28:54-81.

ROCKY MOUNTAIN HERBARIUM STUDIES. VI¹

Aven Nelson

THIS SERIES of papers extends over a considerable period of time—so many years that a brief explanation may be fitting. The first paper (I) appeared in 1931; the second (II) in 1934; the third (III) in 1935; the fourth (IV) in 1936; the fifth (V) in 1938; and the sixth (VI) is now offered. The long intervals between may be accounted for by the fact that the field trips supplying the material for study could not be controlled either as to time or place. Other interests often had to have priority. For this one it happened that studies were begun but often had to be laid aside until further material became available.

The present paper (VI) contains miscellaneous species acquired on several trips into the Southwest (New Mexico, Utah, Nevada and Arizona). The main part of the paper, however, represents collections made in Mt. McKinley National Park, Alaska. In 1939, Mrs. Nelson and I spent the sum-

mer in the Park, commissioned as "Collaborators" with the National Park Service. Because of the assistance given to us by the officers of the Park, we were able to make extensive collections, upon which we were to make a report. Mrs. Nelson (Ruth Ashton Nelson) has prepared that report. It is filed with the Director of the National Park Service, Washington, for possible further attention after the war. In the meantime, sets of the specimens secured have been distributed as follows:

- Set No. 1. The U. S. National Herbarium.
- Set No. 2. The National Park Service.
- Set No. 3. The Rocky Mountain Herbarium.
- Set No. 4. The University of Alaska, Fairbanks.

The few remaining smaller sets have been placed in certain other important herbaria.

All types are in Ry. Mt. Herb.

BETULA beeniana sp. nov.

Arbor, 7-8 m. alta; ramis ramulisque intense brunneis conspicue ceriferoglandulosis haud pubes-

¹ Received for publication December 8, 1944.

centibus; foliis glabris vel margine obscure glanduliferis, suborbiculatis basi late cuneatis apice late obtusis usque 2.5 cm. longis, crenatodentatis, utrinque prominenter nervosis; strobilis ad 2.5 cm. longis 4-6 mm. crassis; squamis valde 3-dentatis mediocriter pubescentibus; nuculis ovalibus fere, 1.5 mm. latis, ala paullo angustiore cinctis.

A small slender tree, to 7 or 8 m. high; twigs and branchlets dark brown, densely glandular but not pubescent, coated with fragile glaucescent waxy film, soon riven and finally falling away as thin flakes; bark on trunk and older branches ragged on the surface with large thin grayish papery flakes exposing the smooth yellowish-brown bark proper, decorated with large conspicuous lenticels, bark not readily separable further into thin layers; leaves firm, glabrous, orbicular in general outline, always broadly obtuse but often pointed by the terminal tooth, broadly cuneate at base, length to 25 mm., the smaller ones sometimes broader than long, margin crenate-dentate, the low obtuse teeth with pale margin that may bear a few inconspicuous gland-tipped hairs; venation conspicuous, raised above the surface on both faces, two main pairs and the mid-rib, breaking up into veinlets toward the tip; fruiting aments to 25 mm. long, 4-6 mm. thick; bractlets 2-3 mm. long, pubescent but not conspicuously so, incisedly 3-toothed, terminal one narrower and longer than the ovate lateral ones, nutlets nearly oval, 1.5 mm. broad, a little broader than the wings.

This proposed species was observed in only one area in McKinley National Park, Alaska. The specimens were taken from a grove of scattering trees on a ridge hillside, in the open spruce woods adjoining the Park Headquarters, at the junction of Rock Creek and one branch of Tanana River. No. 4041, August 7, 1939.

Betula fontinalis Sargt. has been listed from Alaska but probably not from McKinley Park. Furthermore this could scarcely have been mistaken for that species. *B. beeniana* resembles *B. glandulosa* in leaf and twig characters but not in size nor in fruit characters. Since some hybrids have been assumed with *B. glandulosa* as one of the parents, it is possible that *B. ermani* × *B. glandulosa*, listed by Edith Scamman, in her "Plants from Interior Alaska" (Rhodora 42: 321, 1940) may be the species here described. Just why the Siberian species, *B. ermani*, is chosen as the other parent is not evident. Without experimental work, identifying the parents in a cross is an assumption backed up only by varying amounts of circumstantial evidence. This plant needs a name in order to call attention to it as an element in the Alaska flora.

This fine birch is named in honor of Mr. Frank T. Been,² the Superintendent of Mt. McKinley National Park. As "Collaborators" with the National Park Service in the study of the flora of the Park, Mrs. Nelson and I are deeply indebted to Mr. Been for the courtesies and substantial help

² Description and notes prepared in 1940.

that made it possible to carry on the work we had undertaken. Not only the Superintendent and Mrs. Been but all the members of his staff were deeply interested in our studies and gave us encouragement and friendly help.

CALYPTRIDIMUM depressum sp. nov.

Planta prostrata 5-10 cm. lata; caulibus dense foliatis, foliis numerosissimis oblongo-spatulatis; cymis 3-7-floris; sepalis 3-4 mm. longis, ovatis subacutis herbaceis haud albo-marginatis; petalis 2(?); staminibus 2 (1-3); seminibus nitidis.

Depressed prostrate annual, forming dense rosettes from 5-10 cm. broad; stems shorter than, or equalling the numerous oblanceolate-spatulate crowded petioled green leaves; cymes numerous, crowded among the leaves, few-flowered (3-7) and therefore scarcely scorpioid; sepals 3-4 mm. long, subacute, ovate, green, the thin narrow margin pale green (not white); petals always(?) 2, closely enfolding the capsule; stamens 1-3 (usually 2); stigmas nearly or quite sessile; capsule usually a little longer than the sepals; seeds 1-20, black and shining.

This new member of this small southwestern genus is related to both *C. roseum* Wats. and *C. monandrum* Nutt. In both of these the stems are definite and evident. *C. roseum* is further set apart by its conspicuous white-margined sepals and *C. monandrum* by its long slender capsule. The other three or four known species fall into another section and need not be considered here, but each one of them, in one or more characters, stands quite apart from *C. depressum* which can be recognized at once by its completely prostrate habit. It looks like a small green flat mat.

Observed but once, on dry sandy bars, near a little stream at Massacre Camp, in the Pena Blanca Mts., between Ruby, Arizona, and the Tucson-Nogales Highway. Type collection, 1203, Ruth and Aven Nelson, March 15, 1935.

ARENARIA ovalifolia (Hook) comb. nov.

Stellaria ovalifolia Hook. Fl. Bor. Am. 1: 97, 1807.

This species apparently has not been transferred into *Arenaria*. In the North American Flora, Torr. and Gray, 1: 674 and in Syn. Fl. 1: 239 (Dr. Robinson) it is cited as a synonym of *Merckia physodes*. Since Hooker in his Flora recognized both species it is clear that he considered them distinct. In this he certainly was fully justified as shown by specimens when placed side by side. Quoting from descriptions, which are diagnostic, we have the following contrasts:

Stems weak, 12-15 cm. long; leaves pale-green, broadly oval, thin, acute at both ends, much shorter than the internodes; peduncle about 25 mm. long with a single large solitary flower; fruits at maturity 10-12 mm. broad, the carpels inflated.....*A. physodes*.
Stems erect (not weak); leaves broadly oblong, subsessile, green, often purplish (the calyx normally purplish), often as long as the internodes; flowers solitary

or with additional one or two from the upper axils; fruits much smaller, the carpels not inflated

A. ovalifolia

The original specimens of *A. physodes* were collected by Lay and Collie, Capt. Beechey's Voyage, Kotzebue Sound. More recently collected by Mrs. John W. Chapman, from near the Mission, at Anvik, on the lower Yukon, 1924, her No. 15.

A. physodes was secured, in good condition, by Aven and Ruth Nelson, No. 4207, at Cantwell, near the border of Mt. McKinley National Park, Alaska, on the Alaska Railroad grade, August 18, 1939. Secured by A. E. Porsild also, in 1937 at several places in the interior. (See *Rhodora* 41: 229, 1939.) Porsild mentions also "Behring Sea shores, often in brackish meadows," though these latter plants may well have been *A. ovalifolia*.

ACONITUM nivatum sp. nov.

Herba humilis, caulibus 1-2 dm. altis erectis simplicibus vel ramosis e radice tuberiformi; foliis glabris plerumque ad caulis basim restrictis, inferioribus longe petiolatis 3-5-partitis, segmentis inciso-pinnatis, lobulis linearioblongis, superioribus gradatim reductis; floribus solitariis atrocaeruleis, galea circa 2 cm. longa, rostro brevi; filamentis valde alatis; folliculis 3 erectis.

Dwarf, 1-2 dm. high, from a small irregular tuber; tuber oval, oblong, or obconical, less than 1 cm. thick, sometimes tapering into a short, fleshy taproot; roots numerous, dark, long, slender, spreading; stems slender single and simple or stouter and branched; lower internodes short, gradually longer upwards; leaves glabrous, alternate, mostly from the 2 or 3 lower internodes, long-petioled, the blades parted into 3-5 primary divisions which are pinnately cleft into linear-oblong lobes; the uppermost reduced and passing into linear bracts; flowers large, solitary, terminal on the main axis and with 1 or more racemously disposed on axillary peduncles; flowers dark-blue; galea short-beaked, about 2 cm. long, lateral sepals orbicular, almost as long as the galea, about half as wide, stamens numerous, filaments notably wing-margined; carpels mostly 3, erect with short hooked beak.

A near relative of *A. delphinifolium* but not, in my judgment, "a depauperate form" of it. It seems distinct by reason of its small size, roughened tuber concealed in the mass of its dark slender tangled roots, its leaves, which appear to be almost basal, the usually solitary, disproportionally large flower on the nearly naked wand-like stem and by its habitat. *A. delphinifolium* doubtless produces "depauperate" forms but the plant here described normally is as uniform in its habitat as is the other in its habitat. *A. nivatum* is subalpine on sunny open slopes and especially in gravelly slide rock and shale.

No. 4112, Aven and Ruth Nelson, gravel-rock slides, near Camp Eilson, mile 66, Mt. McKinley National Park, August 12, 1939, is taken as the

type, though number 3645, by F. A. Warren, July 3, at mile 64, is equally representative. The difference in dates in these collections simply shows approximately the dates when the snow-drifts disappeared in different exposures. 4077 was secured on Sable Pass, at 4000 ft., August 8, No. 3783, July 14, on Polychrome Pass.

DELPHINIUM alatum sp. nov.

Caulibus ad 1 m. altis simplicibus vel paullo ramosis supra vel ad apicem minute puberulis, racemis pedunculatis obscure glandulosis; foliis praesertim ad medium caulis restrictis 4-5-partitis, segmentis plus minusve divis; floribus intense caeruleis; folliculis approximatis erectis obscure pubescentibus haud glandulosis; seminibus alatis.

With the general aspect of the *D. scopulorum* group, but most closely resembling *D. subalpinum* (Gray) A. Nels., 1 m. or less high, sometimes sparingly branched above, glabrous below but finely puberulent upward and obscurely glandular in the pedunculate spike-like crowded raceme; leaves mostly in the mid-stem region, of 4 or 5 divisions which are variously cleft or divided; flowers dark blue on short pedicels, the spike of erect fruits crowded, the carpels obscurely pubescent but not glandular; seeds with membranous wings half as wide as the body.

Curiously enough, nothing is said in the generic descriptions, nor in the figures generally available, of winged seeds in this genus. Winged seeds do occur—probably in all of the members of the *D. scopulorum* group. In *D. alatum*, they are not easily overlooked. In the field, *D. alatum* suggests *D. subalpinum* (Gray) A. Nels.³ but it lacks the dense yellow glandular pubescence of that species and the lobes of the more divided leaves are narrower. *D. subalpinum* is abundant in, and is characteristic of the Central Rocky Mountains, but its limits northward are not yet definitely determined.

Collection of *D. alatum*: Aven and Ruth Nelson, No. 4093 (*Type*), August 10, 1939, on the Toklat River, near the Alaska Road Commission cabin, Mt. McKinley National Park, wet grass lands with scattering shrubs.

DELPHINIUM hookeri nom. nov.

D. exaltatum Hook. Fl. Bor. Am. 1: 25, 1829. Not *D. exaltatum* Ait. Hort. Kew 2: 244, 1780; nor either of the following, to which it is sometimes referred: *D. scopulorum* Gray, Pl. Wright, 2: 9, 1853; *D. californicum* T. and G. Fl. N. Am. 1: 31, 1838; nor *D. barbeyi* Huth, Bull. Herb. Boiss 1: 335, 1893.

In general aspect, *D. hookeri* resembles *D. barbeyi* but lacks the glandular pubescence of that species. Three collections of *D. hookeri* were secured in Mt. McKinley National Park as follows: No. 3619, near the Park headquarters, steep banks, south end of the railroad bridge, July 1; No. 3788, Igloo Creek Camp, July 10; No. 3984, near Camp Eilson, at "Mile 66," July 25, 1939.

DELPHINIUM nutans sp. nov.

³ *D. barbeyi* Huth (?).

Herba perennis; caulibus 3–4 dm. altis erectis vel assurgentibus leviter pilosis, e radice subliguosa, petiolis foliisque ad nervos et margine paullo pubescentibus; foliis 3–5-partitis, segmentis plus minusve dentatis 2–3-divisis; pedicellis divaricato-ascendentibus; floribus nutantibus, inferioribus foliaceobracteatis remotis; sepalis pedicellis rachibusque paullo pubescentibus, floribus folliculisque etiam obscure glandulosis.

Roots semi-ligneous, fascicled and more or less friable; stems clustered, 3–4 dm. high, erect or assurgent, lightly pubescent with soft white hairs; leaves green but lightly pubescent on petioles, the margins and the veins; blade broader than long, parted into 3–5 divisions, each of which are 2–3 cleft and these lobes irregularly and deeply few toothed; raceme few-flowered, open, except at summit; the lower pedicels 2–3 times as long as the flowers, flexed-ascending, strongly curved outward at summit with the flower nodding and the spur more or less upturned; the short few-flowered racemes at first crowded, becoming open as the linearbracteate pedicels lengthen, usually also a few (2–4) flowers axillary in the uppermost leaves; sepals, pedicels and rachis lightly pubescent; pubescence on the hypanthium and the short thick follicles denser and obscurely glandular.

Aven and Ruth Nelson, in a steep hillside meadow, in the Kantishna mining district, just outside McKinley Park, July 21, 1939. *Type* No. 3910, Rocky Mountain Herbarium.

DELPHINIUM ruthae sp. nov.

Herba humilis; caulibus 10–16 cm. altis e radice valida, plus minusve molliter pubescentibus imprimis ad apicem; foliis omnibus petiolatis plus minusve partitis vel divis, segmentis linearibus vel anguste oblongis, obtusis vel subacutis 1–3 cm. longis supra glabris; racemis albo-pilosis densis, floribus atrocaeruleis, petalis plus minusve albestriatis.

Low and of bushy aspect, 10–16 cm. high; root-stocks stout, dark with dead exfoliating leaf-bases, position determined by the shale rocks from among which they emerge with one or two crowns, each giving rise to only 1 stem; pubescence white and soft, nearly wanting on upper surface of leaves, more noticeable in the inflorescence, dense and subglandular on the carpels; leaves all petioled, the lower with sheathing base, all variously parted and cleft into narrow divisions varying from linear to narrowly oblong, these obtuse or subacute and 1–3 cm. long; inflorescence short, crowded; flowers large, on pedicels equalling them or shorter, beautifully deep-blue, the petals lighter-colored and often streaked with white.

This fine alpine species was observed and collected in two localities only, both times well above timber-line, between 1100–1300 m., in steep slide-rock, on wind-swept slopes. Its root-stocks followed the crevices between the shales. Ruth Ashton Nelson, No. 4052 (*Type*), above Sable Pass, near mile-post 44, McKinley Park, August 8, 1939.

Aven and Ruth Nelson, No. 4108, high slope, near Camp Eilson, Mile 66, McKinley Park, August 14, 1939. In her eagerness for the specimens, Mrs. Nelson, amidst snow flurries and in winds of gale-like proportions, ventured upon the slippery rocks when every step threatened to start a "slide" into the valley.

THLASPI australe sp. nov.

Herba annua glabra; caulibus erectis gracilibus 1–2 dm. altis; foliis subchartaceis inferioribus elliptico-obovatis 8–15 mm. longis, superioribus oblongo-lanceolatis 10–15 mm. longis; pedunculis 2–4 cm. longis; floribus densis albis; sepalis oblongis obtusis late scarioso-marginatis circa 3 mm. longis; petalis late spatulatis 6–8 mm. longis; pedicellis fructiferis usque 24 mm. longis; siliculis 6–8 mm. longis anguste cuneatis, apice rotundatis vel truncatis plerumque 1–2-dentatis.

Annual, glabrous, slender, erect, 1–2 dm. high; leaves open-rosulate at base and alternate on the stem, entire or nearly so, thin, oval to obovate, becoming narrower upward, the uppermost oblong-lanceolate, the blades of the rosulate ones 8–15 mm. long, on slender petioles longer than the blade, the stem leaves narrower, sessile, 10–15 mm. long, auriculate-clasping; peduncle 2–4 cm. long; flowers white, crowded; sepals oblong, obtuse, pale-green with broad scarious margins, about 3 mm. long; petals broadly spatulate, 6–8 mm. long; stamens about half as long, the outer pair as long as the inner; style about 1.5 mm., as long as the ovary, in fruit a little longer; silicle 6–8 mm. long, the pedicels 1–3 times as long, thin and flat, definitely narrowly-cuneate, rounded or truncate at apex and often with 1 or 2 small teeth; seeds two in each cell.

In Dr. Payson's study of the genus in North America (Univ. of Wyoming Publication Bot. 1⁶: 145. 1926) he recognizes six species, besides two varieties of *T. glaucum*. *T. australe* must be compared with the typical form of *P. glaucum* but it differs from that species as follows: it is, to all appearances definitely an annual; its leaves are thin and glaucous, the raceme is pedunculate as in var. *hesperium*, its stem leaves are narrow and exceed the internodes, its pedicels become deflexed, its silicle is at least twice as long as wide.

On the west slope of Baboquivari Peak, Arizona, April 16, 1935 (our No. 1545), by Prof. Leslie N. Gooding, for many years on the research staff of the U. S. Department of Agriculture and always a discriminating collector and student of the Western Flora (*Type*: Ry. Mt. Herb.).

THLASPI prolixum sp. nov.

Herba perennis; caulibus patentibus 12–25 cm. longis; foliis radicalibus integris vel obscure undulatis 10–15 mm. longis, superioribus quam internodiis brevioribus ovato-oblongis; racemis fructiferis densis 4–8 cm. longis; siliculis obovato-cuneatis truncatis vel emarginatis; stylo 2–3 mm. longo.

Perennial; stems few to many (18 in the type) from the expanded crown, moderately stout and wide-spreading, from 12–25 cm. long (including

the fruiting raceme); crown leaves crowded, their blades entire or obscurely undulate, 10–15 mm. long, on slender petioles as long or longer; stem leaves smaller, ovate to oblong, sessile or clasping, shorter than the internodes but continuing to the base of the raceme; raceme crowded even at maturity, from 4–8 cm. long, some racemes with as many as 50 fully developed fruits; fruits 7–8 mm. long, obovate-cuneate, truncate or emarginate, convex on dorsal side and shallowly concave on the other, both sutures conspicuous (almost carinate), the valves thin-edged, style persistent, 2–3 mm. long; seeds 4 in each cell, ovate and plump, pendent, the septum 2–2.5 mm. broad.

The above description has been drawn wholly from the type, No. 1822, Aven and Ruth Nelson. It was secured in the San Carlos Indian Reservation, about 20 miles west of Rice, Arizona, near the road to the mesa and canyon country, May 9, 1935. No flowers were found, but the fruits although quite mature, had not disintegrated in the least.

Other specimens, all mature, are as follows: M. E. Jones, 5374, Marysvale, Utah, June 2, 1894, distributed unnamed but referred by Payson (studies cited above) to *T. fendleri*; Nelson, 10211, Rowe's Well, Grand Canyon, Arizona, April 25, 1925, also doubtfully referred to *T. fendleri*. This would indicate that *T. prolixum* finds its nearest ally in that species.

THLASPI stipitatum sp. nov.

T. Fendleri simillimum, 1–2 dm. altum ubique dense foliatum, foliis radicalibus late ellipticis undulato-dentatis, siliculis stipitatis.

Perennial, 1–2 dm. high, the slender root woody; the crown simple or with one or more very short woody branches; the stems few to several, simple, erect, straight or curved; leaves numerous, crowded on the crowns, nearly or quite overlapping on the stems, the rosulate ones oval to oblong, shallowly undulate-dentate, some of them purple or purplish, 10–15 mm. or more long, on slender petioles usually 2–3 times as long; the lower cauline sometimes short-petioled, but usually sessile and becoming auriculate-clasping, ovate or narrower, 10–15 mm. long, some of them greatly reduced; inflorescence at first crowded and appearing capitate, the axis pedunculate; flowers purple, shading through lengthening in fruit and then seemingly short-lighter to almost white; sepals dark green or purple, oval, obtuse, narrowly white-margined, 2–3 mm. long; petals with oval obtuse blade about 3.5 mm. long, tapering gradually into the stipe-like base of equal length; fruits (not mature) cuneate-oblong, truncate or emarginate at apex, only about 4 mm. long with a style of equal length, tapering abruptly into a stipitate-base 1–2 mm. long; pedicels varying from divergent to reflexed, 1–3 times as long as the fruit.

This cannot be referred to *T. fendleri*, its nearest relative, because of its much larger size, its great leafiness throughout, its large oval dentate basal leaves and the stipitate silicles.

No. 1196 by Aven and Ruth Nelson is the type collection. Secured in the Pena Blanca Mountains, south of Tucson, not far from Ruby, Arizona, March 15, 1935.

LUPINES.—The lupines must be placed among the plants that have given to Alaska its reputation as a land of singular floral beauty. The species are few but their abundance and wide distribution enliven many of its landscapes. This study of the lupines of Alaska, particularly of Mt. McKinley National Park, cannot be concluded until I return to my home herbarium and library in June. However, one new species may be published now.

LUPINUS toklatensis sp. nov.

Planta fere glabra vel sparse pilosa; caulibus erectis 3–4 dm. altis; foliis radicalibus numerosis, petiolis 8–12 cm. longis, stipulis lanceolatis acutis; foliolis plerumque 8 oblongis vel oblanceolatis utrinque angustatis ad 3–4 cm. longis; pedicellis 3–5 mm. longis plerumque verticillatis; floribus caeruleis glabris, circa 12–14 mm. longis; legumini-bus dense albo-villosis.

Plant sparsely soft-pubescent or nearly glabrous; root stout, woody, with several crowns; stems several or numerous, erect, 3–4 dm. high (including the raceme); basal leaves numerous, on slender petioles 8–12 cm. long; cauline similar on gradually shorter petioles; stipules lanceolate, acute; leaflets mostly eight, oblong to oblanceolate, tapering to the acute apex and to the slender base, up to 3–4 cm. long; inflorescence large and heavy in proportion to the stem, up to 10 cm. long, surpassing the leaves; flowers large, crowded, on short (3–5 mm.) pubescent pedicels, mostly verticillate; flowers blue, wholly glabrous; banner orbicular, 12–14 mm. long, blue with a conspicuous white stripe down the middle including the v-shaped fold (the white stripe turns brown in drying); wings obovate, about as long as the banner; keel narrow, a little shorter, all the parts nearly clawless; pod densely white-villous; ovules few, only 3–4 maturing.

Secured in the Toklat area only, Mt. McKinley National Park, Alaska. Near the Park highway, mile post 56, at the Toklat cabin, fls. June 25, 1939, no. 3568; fr. August 27, no. 3568a. Type in U. S. Natl. Herb.; iso-type Ry. Mt. Herb., Univ. of Wyoming.

AMSONIA biformis sp. nov.

Herbacea perennis ubique glabris vel molliter puberulis; foliis linearilanceolatis; lobis calycis piloso-ciliatis 5–7 mm. longis; corollae lobis albis vel plus minusve purpureis tubo 7–9 mm. longo; stigmatibus obscure lobatis.

Perennial with few to several stems from the woody crown, 3–5 dm. high, the plants occurring in two forms under identical conditions: some individuals green and wholly glabrous throughout; others grayish-green throughout with a soft uniform puberulence; leaves numerous, lance-linear, short-petiolate; inflorescence borne well above the leaves, crowded-subcapitate, flowers many (20–30 or more); calyx lobes narrowly linear, softly cili-

ate on the margins, 5–7 mm. long; corolla 2 cm. or more long, very variable in color, its lobes purplish-blue to white, usually with a yellowish tinge at base, its slender tube bluish-brown or dark purplish-green, the tube proper 7–9 mm. long and above a constriction the expanded throat almost equally as long; pubescence dense and soft just below the constriction, sparse downward, anthers and stigma almost free, corolla lobes oblong-ovate, 6–7 mm. long; stigma small and subcapitate but the three areas evident, the lobes very short with scarcely more than a groove between them.

The two forms to which the name refers are inseparable except for the uniform pubescence of one of them. This was so evident that one could sit in the patch and, without rising, spot them readily and gather both forms from among the broken black lava blocks that covered the base of the low mountain.

It is probably to be included in the same section as *A. macrantha* and *A. pogonosepala* but the characters, as given seem to preclude its reference to either of them.

Different numbers were given to the two forms but the material is made into specimens carrying both forms, so that the number may be written 11278–79. Secured several miles west of Duncan, Arizona, in the foothills north of the Duncan-Safford highway, by Aven Nelson, March 22, 1930.

ERIGERON denalii sp. nov.

Herbaceus, rhizomatosus; caulibus foliosis ad basin, scapiformibus monocephalis plus minusve bracteatis erectis 7–14 cm. altis; foliis integerrimis anguste oblanceolatis 1.5–3 cm. longis in petiolum brevem attenuatis; involucris 9–12 mm. latis hirsutis vel ad basin sublanatis; squamis lineari-acuminatis 6–8 mm. longis, purpureo-viridibus; corollis florum radii ligulatis circa 40, ligulis subpurpureis 6–8 mm. longis, disci tubulosis, tubulo circa 3 mm. longo limbum subaequante, limbo ad apicem roseo-vel purpureo-colorato; pappi setis subrigidulis circa 3 mm. longis; achaeniis denium clavatis firmis glabris circa 3 mm. longis.

Rootstocks slender, simple or few branched; stems short, leafy at base, 1–3 cm. long, the scapose-like, bracted monocephalous erect peduncle 7–14 cm. high; leaves entire, mostly narrowly oblanceolate, 1.5–3 cm. long, tapering to a narrow petiolar base, those of the peduncle few (or wanting) and bract-like; involucre 9–12 mm. broad, hirsute, often sublanate at base; phyllaries numerous, linearacuminate, 6–8 mm. long, purplish-green; rays about 40, 6–8 mm. long, lavender; disk flowers slender, tube and throat subequal, teeth and throat rose or purple; pappus coarse and abundant, in a single series, rose-colored; achene when fully mature clavate, firm, smooth and essentially glabrous; achene, pappus and tube of corolla each about 3 mm. long.

Dr. E. L. Greene (Pitt. 4: 155. 1900) described *Erigeron purpuratus*, from the Yukon. This he compares to *E. compositus* as to its caespitose habit

and the early rosulate leaves with dilated, 3-toothed or 3-lobed tips. In the absence of any reference to color of disk flowers, one may assume they are yellow as in the genus *Erigeron* in general. We secured plants meeting this description perfectly, our number 4014, from near McKinley Park, R. R. Station, gravelly soil on banks near the Nenana River. While the plant here described is definitely related to Dr. Greene's plant, its aspect is so different and the rose-purple-lavender color so pronounced throughout, and the achenes so strongly characteristic, it seems best to consider it as another, more divergent, member of the *E. compositus* alliance. Greene's species, in common with the other *E. compositus* relatives, is caespitose on a tap-root while *E. denalii* arises from running rootstocks.

The collections are Aven and Ruth A. Nelson, No. 4058 (Type), Sable Pass, where the Park road reaches its highest point (about 4000 ft.); August 8, 1939. No. 4016, upper Savage River, August 3, 1939. A single plant, wholly typical, was brought to us by Mr. Harold Herning out of McGonigle Pass, August 4, 1939.

SENECIO denalii sp. nov.

Herbaceus perennis (?), ubique praesertim ad apicem sparse lanatus; caule erecto 1–1.5 dm. alto foliato plerumque monocephalo; foliis intense viridibus integris radicalibus anguste oblongis subobtusis basi sensim angustatis fere sessilibus eis caulinis gradatim reductis, supremis bracteiformibus; involucris 14–18 mm. latis, bracteis lineari-oblongis circa 8 mm. longis; ligulis 14–16 mm. longis patentibus vel plus minusve refractis; floribus disci numerosis.

From a short dark vertical rootstock with many slender pale roots; sparingly but permanently lanate, especially above; stem simple, slender and erect, 1–1.5 dm. high, monocephalous or rarely a smaller accessory head from the uppermost long-linear foliar bract; leaves few, subglabrous, all entire, dark-green; the crown-leaves narrowly oblong, subobtusate, 2–4 cm. long or more, sessile by a narrowed base; stem leaves similar but more evidently lanate, becoming lanceolate and passing into smaller linear bracts, giving a scapose effect to the stem; heads large, involucre 14–18 mm. broad, the linear phyllaries about 8 mm. long, equalling the disk-flowers; rays long (14–16 mm.), spreading and often irregularly refracted, broadly linear, tapering toward the base, orange to burnt-orange, the three or more nerves conspicuously darker in dried specimens, converging to a darker usually toothed tip; tube of disk flower very slender, about 3 times as long as the throat; achene short (about 2 mm.), pubescent.

The species here proposed is evidently nearest to *Senecio tundricola* Tolm. Comptes Rendus Acad. Sc. U. R. S. S. 266. 1928. A specimen of that is not at hand nor is a description available in American literature. Dr. Eric Hultén, Fl. Kamtch. 206. 1927, gives the name and citations and gives its range as

"? Asiatic, transgressing into W. America." Under area he writes "Arctic America?" He further says "it may be said to come between *S. frigidus* and *S. campestris*." He also ventures the opinion that *S. tundricola* "is identical with *S. integrifolia* var. *lindstromii* Ostenf." Dr. A. E. Porsild, in his paper, Contrb. to the Fl. of Alaska, Rhodora 41: 298-299, does not include *S. tundricola* and he definitely reduces *S. integrifolia* var. *lindstromii* to *S. atropurpureus*. In the face of this uncertainty as to validity of the species *S. tundricola* and the seeming distinctness of *S. denalii* from the known species with which *S. tundricola* has been compared one may well assume the validity of *S. denalii*.

The name *Denali* is the Indian name for Mt. McKinley, the highest peak in North America (20,300 ft.). It dominates the landscape of interior Alaska. To the natives it has always been personified as "The-High-One" (*Denali*), or the High-god.

S. denalii occurs in wet swales in the mountains (2500-3500 ft.), usually in open patches of low-growing caespitose willows. Aven and Ruth A. Nelson, no. 3800 (Type), Polychrome Pass, July 14, 1939; no. 3636, contributed by F. A. Warren, Highway Pass, July 3, 1939.

TARAXACUM carneocoloratum sp. nov.

Planta gracilis; foliis oblongo-lanceolatis 3-6 cm. longis 1-1.5 cm. latis marginibus 3-6-dentatis, dentibus plus minusve ovatis subacutis; scapo 1-1.5 dm. alto apice parce albo-arenoso; capitulis mediocribus; bracteis exterioribus late ovatis 3-5 mm. longis erectis deinde patentibus vel paullo reflexis viridibus vel margine rubro-tinctis, bracteis interioribus graciliter-corniculatis plus minusve roseo-suffusis; ligulis definite carneo-coloratis; acheniis

pallidis circa 4 mm. longis apice solum spinuloso-muricatis.

Perennial, the short tap-root with one or more crowns, each with several leaves and 1 or 2 scapes; glabrous except for a scanty white pubescence at the base of the involucre; leaves oblong-lanceolate in outline, the blade 3-6 cm. long, 1-1.5 cm. broad, the 3-6 pairs of teeth subacute and more or less triangular; scapes 10-15 cm. long, slender; head comparatively large; involucre broadly campanulate, its outer bracts in two series, broadly ovate, the thicker center green becoming tinged with red, at first erect but finally spreading or somewhat reflexed, 3-5 mm. long, inner bracts margined, slender-corniculate, green but gradually becoming pink; flowers definitely flesh-colored from the beginning to maturity; achenes pale, somewhat flattened, about 4 mm. long, spinulose-muricate at tip only, beak slender, longer than the achene, tapering from base to the disk bearing the white pappus which is as long as the beak.

To find a *Taraxacum* that is definitely some other color than yellow was so much of a surprise that some pains were taken to determine that it was not a mere color phase. The pink (flesh color) of the ligules seemed constant. The tendency to redness was emphasized by the redness in the other floral structures.

Two collections were secured but both were in the same general area. First by Fred A. Warren, July 3, 1939, on a wet ridge, near Stony Pass, Mt. McKinley National Park; then by Aven and Ruth Nelson above Stony Pass, near mile-post 61, July 3, 1939, no. 3646 (Type), Rocky Mountain Herbarium.

UNIVERSITY OF WYOMING,
LARAMIE, WYOMING

ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

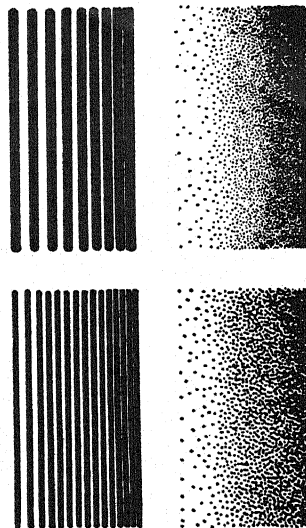
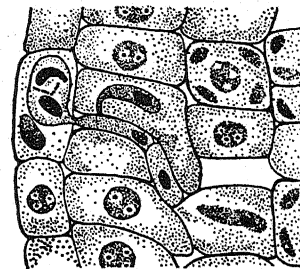
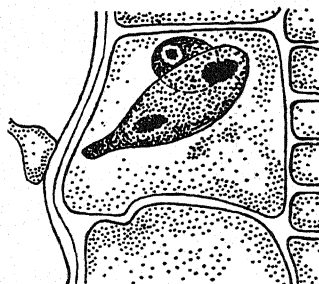
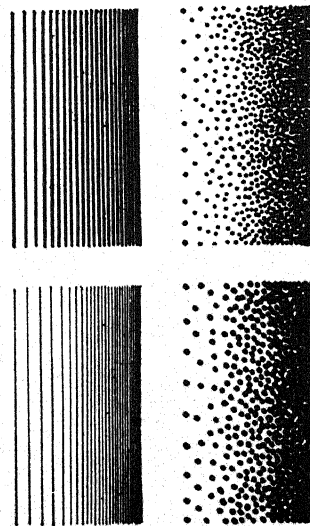


ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to $\frac{1}{4}$. Middle—Reduction to $\frac{1}{2}$. Bottom—Original size.

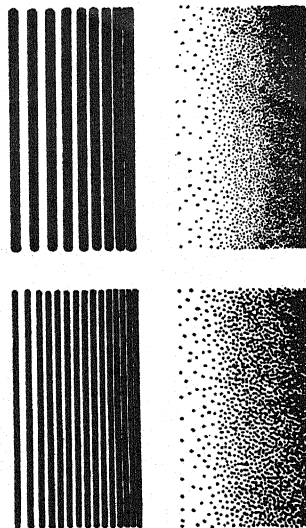
Reprinted by permission of the authors from: RIKER, A. J., and REGINA S. RIKER. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



THE EVOLUTION OF OXYGEN FROM SUSPENSIONS OF CHLOROPLASTS; THE ACTIVITY OF VARIOUS SPECIES AND THE EFFECTS OF PREVIOUS ILLUMINATION OF THE LEAVES¹

Johanna Kumm and C. S. French

THE NUMBER of studies of the physiological activity of isolated chloroplasts is relatively small, for their main function, photosynthesis, does not continue when they are removed from the living cell. The early work with isolated chloroplasts, reviewed by Spoehr (1926) and by Inman (1935), has shown that some oxygen-evolving capacity remains after they are removed from the living cells, but its rate is low relative to photosynthesis of normal leaves and the oxygen appears to come from some substance present in very limited amounts. More recently Menke (1938) and also Comar (1942) have isolated spinach chloroplasts by differential centrifugation and have presented analytical data as to their composition. Granick (1938) has also presented analyses of spinach chloroplasts. Neish (1939) isolated chloroplasts and studied the factors affecting their flocculation. Preparations of oat chloroplasts were analyzed for their nitrogen content by Galston (1943) who also described their agglutination by castor bean extracts.

Hill (1939a and b) has shown that suspensions of macerated leaves which contain intact chloroplasts, and to which ferric salts of an organic acid have been added, evolve oxygen when illuminated. This oxygen was measured manometrically in the presence of potassium ferricyanide by Hill and Scarisbrick (1940b). Such an evolution of oxygen may be similar to or identical with corresponding steps in normal photosynthesis. It is brought about by the reduction of ferric salts without a simultaneous reduction of carbon dioxide and its rate is more nearly comparable to that of photosynthesis in intact leaves. Other aspects of this phenomenon have been described by Hill and Scarisbrick (1940a and c), French and Rabideau (1945), French and Anson (in preparation), and by Holt and French (in preparation). Fan, Stauffer and Umbreit (1943) have furnished additional evidence that the evolution of oxygen and the reduction of carbon dioxide are separate steps in the process of photosynthesis by adding benzaldehyde and other reagents to *Chlorella* cells which then evolved oxygen in the absence of carbon dioxide.

In our work with the "Hill reaction" we were interested in comparative measurements of the capacity of the chloroplasts of various species to produce oxygen, particularly those that could be subjected to different environmental conditions, yet

¹ Received for publication December 4, 1944.

It is a pleasure to thank Professors C. O. Rosendahl and N. L. Huff and Dr. J. W. Moore of this department for their kind assistance in the identification of the plant species.

still remain active. It will be shown that the history of the plant's relation to light prior to chloroplast isolation is at least as important as the species used.

PROCEDURE.—Isolated chloroplasts were prepared in the following way. Leaves were picked from growing plants, and were immediately put into cold tap water. They were thoroughly washed, then placed in a large porcelain evaporating dish containing tap water kept at about 10°C.; to this was added a small amount of potassium bicarbonate. The material was exposed to light in front of a window in the laboratory for one-half to one hour. For those experiments in which the plants had been subjected to specific periods of illumination or of darkness, the leaves were removed, washed, and used without this light exposure period.

Several handfuls of leaves were then placed in a Waring blender with 200 cc. of ice-cold 0.5 M sucrose solution. The blender was operated for one minute after the last leaf was drawn into the liquid. At the end of this time the mixture was poured through a fine textured muslin cloth to remove intact cells and fibers. The liquid was gently squeezed into a beaker kept in a cold water bath at 0°C. This suspension was centrifuged in an angle centrifuge at 2700 r.p.m. for ten minutes. The tubes were refilled with more of the original suspension and replaced for a second centrifugation. The chloroplasts were then collected in a cool test tube in a few cc. of 0.5 M sucrose solution and replaced in the cold water bath until ready for use.

In the main chamber of a double side arm Warburg vessel was placed 2.0 cc. of a modified Hill's solution, containing the following substances: potassium oxalate 0.5 M, ferric ammonium sulfate 0.01 M, potassium ferricyanide 0.02 M, sucrose 0.2 M, borate buffer pH 7.0, 0.167 M. The modifications from Hill's original mixture are those found by Newcomb in unpublished work to give higher rates of O₂ evolution. In one side arm was placed 0.5 cc. of the chloroplast suspension. In directly comparable experiments the amount of the suspension added to the vessels was adjusted by dilution to give the same amount of chlorophyll in all preparations. Above approximately 0.3 mg. chloroplast chlorophyll per vessel, lower activity values are obtained. The manometers were then put in a constant temperature water bath kept at about 5–10°C. After the first 10 minutes of shaking, the manometers were removed and the chloroplast suspension mixed with the rest of the liquid. The manometers were replaced and shaking was resumed for 3 minutes in the dark, after which the light was turned on

from two Lumiline bulbs suspended about 2 cm. below the flasks. This gave a sufficiently bright light so that the rate of the reaction was essentially independent of the intensity. Readings were taken at 5-minute intervals for 30 minutes. The oxygen evolved was plotted against time and the rates calculated graphically from the initial slopes of these curves. The rate of oxygen evolution based on the chlorophyll content (M = mg. chlorophyll per vessel) was calculated as follows:

$$Q_{O_2}^{ch} = \frac{\text{cmm. } O_2}{\text{Hrs.} \times M}$$

Triplicate measurements were made in every experiment throughout our entire work. More detailed accounts of the procedures used are given by French and Anson (in preparation).

EXPERIMENTS AND RESULTS—*Measurements of the activity of chloroplasts from various species.*—Suspensions were prepared in the above manner from various plant species listed in table 1. In each case leaf tissue was obtained from growing specimens except in the case of spinach which was procured at a grocery store.

Much of the previous work has been done on spinach since it can be conveniently purchased the year around. This species did not, however, lend itself easily to a series of light treatments due to some difficulty in cultivating it in our greenhouse. We therefore proceeded to investigate a number of species having chloroplasts that were capable of evolving oxygen and that could be subjected to various conditions. In the search for such a species we found that *Tradescantia*, which is easily propagated and which also has the property of withstanding a number of environmental changes, gave high activity.

Many species tested for oxygen evolution contained tannins or phenolic compounds and when combined with Hill's solution formed a dark color, thus failing to show evidence of activity. Species of plants giving such a reaction were *Rosa rugosa*, *Polygonum aviculare*, *Syringa vulgaris*, *Urtica dioica*, *Ambrosia artemisiifolia* and *Rhus typhina*. A brown precipitate and a severe clumping of chloroplasts occurred in preparations from *Taraxacum palustre* var. *vulgare*, *Urtica dioica*, *Ambrosia artemisiifolia* and *Plantago major*. Suspensions prepared from *Castalia odorata* contained a large number of starch grains and therefore required a number of differential centrifugations in order to remove the starch. After this extended process there was no activity. Other species from which we obtained no activity in a single trial were: *Oplismenus compositus*, *Acer Negundo*, *Oxalis stricta*, *Sedum* sp., *Lilium tigrinum*, *Commelina communis*, and *Zebrina pendula*.

Hill (1939b) reports that *Stellaria media* gives little or no activity after 6:00 p.m., due we believe to the light effect described below. We have no clear-cut evidence in our work that the activity

varies with time of picking during the daylight hours, although such an effect would be a reasonable consequence of the data reported below.

TABLE 1. A comparison of the oxygen evolving capacity of chloroplasts from several species of plants.

Plant	Rate of O_2 evolution	Concentration of chloroplasts	Activity
	cmm. O_2 / 10 min.	mg. chyll. per vessel	$Q_{O_2}^{ch}$
Aster tatarica	38	1.95	120
Aster tatarica	21	1.14	110
Aster tatarica	15	1.65	55
Aster tatarica	25	0.72	210
Aster tatarica	16	0.60	160
Mirabilis jalapa	15	0.46	195
Mirabilis jalapa	14	0.46	180
Mirabilis jalapa	15	0.48	190
Mirabilis jalapa	6.5	0.33	120
Mirabilis jalapa	14	0.48	175
Impatiens biflora.....	16	0.42	230
Impatiens biflora.....	17	0.42	240
Impatiens biflora.....	17	0.41	250
Impatiens biflora.....	18	0.42	260
Impatiens biflora.....	5.5	2.3	14
Impatiens biflora.....	6.5	0.83	47 ^a
Impatiens biflora.....	14	0.42	200 ^a
Impatiens biflora.....	8.2	0.2	250 ^a
Tradescantia fluminensis	24	0.45	320
Tradescantia fluminensis	27	0.45	360
Tradescantia fluminensis	30	0.43	420
Tradescantia fluminensis	42	0.17	1500 ^b
Tradescantia fluminensis	34	0.17	1200 ^c
Spinacia oleracea.....	12	0.83	87
Spinacia oleracea.....	17	0.18	570

^a Diluted with water, chloroplasts still intact.

^b Leaves put in ice water and exposed to window light for two hours.

^c Same suspension as in line above but kept at 0° for 24 hours.

The chloroplasts of various species behave differently during extraction. Some leaf tissues (e.g., *Impatiens*) produce intact chloroplasts withstanding several centrifugations, whereas some, like *Tradescantia fluminensis*, have chloroplasts which break up into fragments in the blender. In the preparation of the material, a 0.5 M sucrose solution was used to maintain an approximately isotonic solution. However in several preparations of *Impatiens*, suspensions which were made as described above and then were diluted with redistilled water nevertheless showed a high activity. Microscopic observations indicated no changes in the structure of chloroplasts in dilutions down to 0.05 M sucrose.

Increase in activity of chloroplasts isolated from plants illuminated for various lengths of time after a long dark period.—Since it is evident from table 1 that a great variation of activity can be obtained from different samples of the same species, as well as by using different species, we attempted to find

the cause of this variation. Since the most obvious variable in the immediate past history of the plants probably had to do with light intensity, we investigated the effects of prior exposure to light or to darkness on the oxygen evolution of the subsequently isolated chloroplasts.

Two series of *Tradescantia* twigs were placed in the dark at 20°C. in gallon jars with wire mesh guides to hold them upright. The lower ends of the plants were in water. The first series was left in the dark for two days and the second for three days. At the end of the dark periods some leaves were picked, the chloroplasts removed, and their activity measured. The plants were then illuminated by tungsten bulbs yielding 150 foot candles after the light had been filtered through seven inches of water. Light exposures of two, four, and six hours were given and, at the end of each of these periods of time, the activity of the chloroplasts was measured again. The experiment was repeated using the same two dark periods, followed by exposure to sunlight of 5,000 f.c. The results of these measurements are given in figure 1 which shows that the activity increases in direct proportion to the time of exposure, to sunlight as well as to the weaker light at least up to six hours. Presumably longer periods would have resulted in still greater activities. A possible interpretation of this effect will be presented below.

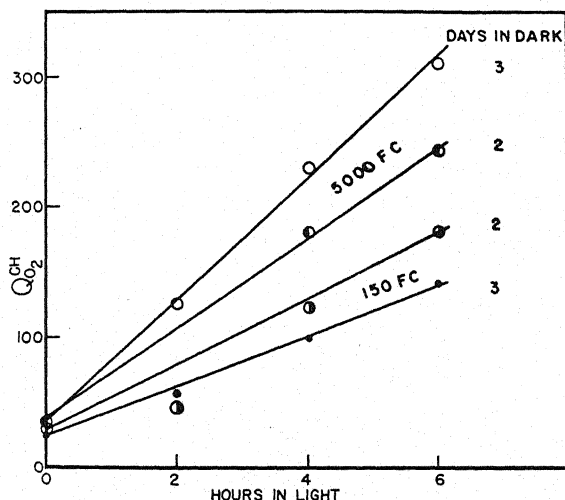


Fig. 1. The increase in rate of O_2 evolution, in mm. O_2 per hour per mg. of chlorophyll, of chloroplasts isolated from plants kept in the dark for two or three days and then exposed to light for various lengths of time prior to removal of the chloroplasts.

We also measured the activity of suspensions from plants that had received light treatments of 72 or 96 hours with light intensities of 150 or 350 foot candles. The purpose of this was to determine whether the intensity of the illumination or the length of time involved was the more important factor. It was observed that the duration of treatment

had a greater effect than the intensity of light. Long exposures to light of 150 f.c. and of 350 f.c. gave nearly identical activities as shown in table 2.

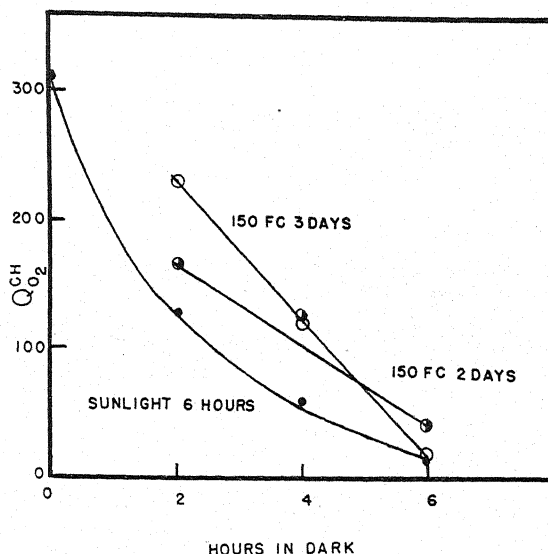


Fig. 2. The decrease in rate of oxygen evolution of chloroplasts isolated from plants kept in the dark for various lengths of time.

At the same time it seemed important to vary the medium in which the plants were cultivated. Two series of plants were arranged, one in tap water and the other in soil, both under tungsten light of 150 f.c. In both, however, the activity was the same. After 96 hours both gave a $Q_{O_2}^{ch}$ of 176.

TABLE 2. The comparative effects of intensity and of time of illumination of plants on the rate of O_2 evolution of chloroplasts subsequently isolated from them.

Time	Rate of O_2 evolution	
	Illumination 150 f.c.	Illumination 350 f.c.
(hrs.)	$Q_{O_2}^{ch}$	$Q_{O_2}^{ch}$
72	275	282
96	368	362

Decrease in activity of chloroplasts isolated from plants kept in the dark for various periods.—The activity of chloroplasts taken from plants which had been kept in the dark for various lengths of time was measured. In some experiments, *Tradescantia* plants were taken from the greenhouse after having received six hours of sunlight and been placed in the dark. In other experiments, plants were placed in the dark after having been kept at 20°C. under 150 f.c. of water-filtered tungsten light, one series for two days and another for three days. Chloroplast suspensions from these specimens were tested at intervals. The results are shown in figure 2. The data are probably not precise enough to give great

significance to the shape of the curves, but it is evident that the decrease in activity is rapid. The curve for the plants previously in sunlight dropped to one-half of its initial height in 1.6 hours. It is obvious from these data that experiments on the Hill reaction should be made with chloroplasts from plants that have been previously exposed to light for a considerable length of time.

Effects of illumination on the retention of activity of chloroplasts suspended in sucrose at 5°C.—Since illumination of plants prior to the extraction of chloroplasts increased the activity of the isolated chloroplasts, an attempt was made to see if the same effect could be observed in chloroplasts removed from the leaves and irradiated when suspended in sucrose solution. No increase in activity was found as a result of storage of extracted chloroplasts in the light, but the loss of potency on standing was considerably reduced.

For this experiment chloroplasts isolated from *Tradescantia* were treated with water-filtered light from a tungsten lamp. The light was reflected from an elliptical mirror placed in such a position as to allow a beam of light of 3,000 f.c. to fall on the suspension spread as a thin layer in a Petri dish placed in a water bath at 5°C. The dark controls were kept in the water bath in wrapped test tubes. After four and six hours of illumination samples were taken for activity measurements. The oxygen released from the illuminated suspensions remained fairly constant for this time and was higher than the activity of the control suspension kept in the dark. The results are presented in table 3. Since the dark control was kept in a test tube instead of in a Petri dish, the difference in aeration may have had some effect.

TABLE 3. Activity of *Tradescantia* chloroplasts stored at 5°C., with and without illumination of 3,000 f.c.

Time of storage	Dark control	Illuminated
(hrs.)	$Q_{O_2}^{\text{ch}}$	$Q_{O_2}^{\text{ch}}$
Experiment 1		
0	660	660
2	510	650
4	420	640
Experiment 2		
0	640	640
4	330	640
6	390	560

It is to be noted that the loss of activity in *Tradescantia* during dark storage was much greater in this experiment than in the one shown in table 1, or than in other experiments using *Impatiens biflora*. The chlorophyll concentration in the vessels used for activity measurements at 8°C. was 0.38 mg. per vessel.

DISCUSSION.—*Variation of activity in relation to species.*—If we pick out of table 1 the values for the activities measured at the lower chloroplast concentrations only, we obtain the following $Q_{O_2}^{\text{ch}}$

values: *Aster tatarica* 160–210, *Mirabilis jalapa* 120–180, *Impatiens biflora* 200–260, *Tradescantia fluminensis* 320–1,500, *Spinacia oleracea* 570 (other spinach data in this laboratory give a usual range for many measurements over a three-year period of 100–700). The high *Tradescantia* result was obtained from plants that had been longer illuminated and had been kept cooler than the rest of the plant material used. In other work (French and Rabindeau, 1945), it was found that the efficiency at low light intensity was similar in both *Tradescantia* and spinach. Except for those plants which have interfering substances causing entire loss of activity, it would seem from this brief survey that differences in activity among the preparations from usable plants are not significantly correlated with species.

On the basis of the data so far available we might tentatively consider the Hill reaction to be a phenomenon generally associated with the usual type of photosynthesis mechanism common to all green plants, except those that contain the interfering substances already described by Hill. The inability of intact leaf and algal cells to show this reaction may possibly be caused by impermeability of the cell wall to the reagents used. That this is more likely than the presence of cytoplasmic inhibitors is evident from the fact that Hill used crude leaf macerates with success and that we have generally found activity in such preparations from spinach.

The effect of prior illumination of the leaves.—The increased activity of chloroplasts from plants that have been exposed to light before chloroplast isolation may be a reflection of what may be called loosely the physiological state of the leaf. A more specific interpretation that we have adopted as a working hypothesis is that the light exposure periods builds up, by means of normal photosynthesis, the concentration of some unidentified substance, which in addition to the Hill reagents and the components of dark-adapted chloroplasts is essential for the evolution of O_2 . It is tempting to think of this hypothetical substance as an intermediate in the usual process of photosynthesis which increases in concentration in the light and is then slowly removed by a dark reaction. If this does turn out to be the case we might be able to measure its concentration in plant extracts by following the acceleration of the rate of O_2 evolution of dark-adapted or of washed chloroplasts when extracts containing this substance are added to it. So far we have little idea of the maximum rate that may be attained by a prolonged preliminary exposure to light. Up to six hours there is no apparent falling off of the stimulating effect of light. One experiment with *Tradescantia* gave a $Q_{O_2}^{\text{ch}}$ of 1,500, about five times the maximum attained in the results shown in figure 1, suggesting that the effect may be even greater than has yet been found in any measurements with adequate dark controls.

Since in these experiments bright light was used, the rate of the reaction is independent of the light

intensity, that is to say, the measured rate is determined by the maximum rate attainable by the enzymatic system rather than by the rate of the photochemical step. It is therefore not known if the photochemical step is or is not influenced by the previous illumination of the leaves. If such were the case this might have considerable bearing on the variability found in the quantum yield determinations of the Hill reaction described by French and Rabindeau (1945).

Many of the experiments reported in table 1 were made with too high a chloroplast concentration in the vessels to give reliable QO_2^{ch} values and they should not be taken to indicate low activity of those preparations. A few measurements were undertaken to construct a curve from which correction factors could be deduced to reduce all the data to a low chloroplast concentration, but we believe that for the present data comparisons at about 0.4 mg. chloroplast chlorophyll per vessel will give as satisfactory results. Curves relating rate and chloroplast concentration will be given elsewhere. The present data are not of a high order of accuracy since we do not have any comparisons of replicate extractions from the same batch of leaves. The general consistency of the data of figure 1 does however suggest that there is no great variation introduced by the extraction procedure.

SUMMARY

The evolution of oxygen from illuminated suspensions of chloroplasts in solutions containing ferric oxalate and potassium ferricyanide was measured using chloroplasts from twenty-two different species of higher plants. Many gave no reaction whatever, due to the presence of interfering substances, presumably tannins, that combine with the added iron to form highly colored compounds. Those species that do give the oxygen evolution reaction have roughly similar ranges of activity based on the amount of chlorophyll present. Various preparations of the same species gave as different activities as did preparations from assorted species. This may be due to the past history of the specimens, particularly in regard to their exposure to light, which was found to increase the rate of oxygen evolution markedly as compared to that from plants kept in the dark before removal of the chloroplasts. This increased activity of light-treated plants dies away again when they are stored in the dark. The light effect is suggested as being due to the accumulation of some unidentified intermediate of normal photosynthesis.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MINNESOTA,
MINNEAPOLIS, MINNESOTA

LITERATURE CITED

- COMAR, E. L. 1942. Chloroplast substance of spinach leaves. *Bot. Gaz.* 104: 122-127.
- FAN, C. S., J. F. STAUFFER, AND W. W. UMBREIT. 1943. An experimental separation of oxygen liberation from carbon dioxide fixation in photosynthesis by *Chlorella*. *Jour. Gen. Physiol.* 27: 15-28.
- FRENCH, C. S., AND G. S. RABIDEAU. 1945. The quantum yield of oxygen production by chloroplasts suspended in solutions containing ferric oxalate. *Jour. Gen. Physiol.* 28: 329-342.
- GALSTON, ARTHUR W. 1943. The isolation, agglutination and nitrogen analysis of intact oat chloroplasts. *Amer. Jour. Bot.* 30: 331-334.
- GRANICK, SAM. 1938. Quantitative isolation of chloroplasts from higher plants. *Amer. Jour. Bot.* 25: 558-561.
- . 1938. Chloroplast nitrogen of some higher plants. *Amer. Jour. Bot.* 25: 561-567.
- HILL, R. 1939a. Oxygen evolution by isolated chloroplasts. *Nature* 139: 881.
- . 1939b. Oxygen produced by isolated chloroplasts. *Proc. Roy. Soc. London B* 127: 192-210.
- , AND R. SCARISBRICK. 1940a. The reduction of ferric oxalate by isolated chloroplasts. *Proc. Roy. Soc. London B* 129: 238-255.
- , AND ———. 1940b. Production of oxygen by illuminated chloroplasts. *Nature* 146: 61-62.
- , AND ———. 1940c. The reduction of ferric oxalate by isolated chloroplasts. *Proc. Roy. Soc. London B* 129, S 39. (Abstract.)
- INMAN, O. L. 1935. The evolution of oxygen in the process of photosynthesis. *Cold Spring Harbor Symposia on Quantitative Biology* 3: 184-190.
- MENKE, W. 1938. Untersuchungen über das Protoplasma grüner Pflanzenzellen 1. Isolierung von Chloroplasten aus Spinatblättern. *Hoppe-Seylers Zeits. f. Physiolog. Chemie.* 257: 43-48.
- NEISH, A. C. 1939. Studies on chloroplasts 1. Separation of chloroplasts, a study of factors affecting their flocculation and the calculation of the chloroplast content of leaf tissue from chemical analysis. *Biochem. Jour.* 33: 293-299.
- . 1939. Studies on chloroplasts 2. Their chemical composition and the distribution of certain metabolites between the chloroplasts and the remainder of the leaf. *Biochem. Jour.* 33: 300-309.
- SPOEHR, H. A. 1926. *Photosynthesis*. A.C.S. Monograph No. 29 Chemical Catalogue Co. New York.

NUTRIENT REQUIREMENTS IN THE GERMINATION OF THE CONIDIA OF GLOMERELLA CINGULATA¹

Ch'wan-Kwang Lin

IN A previous work (1940) the writer showed that the conidia of *Sclerotinia fructicola* (Wint.) Rhem require nothing but an energy material for germination. It was thought that the same might hold true for *Glomerella cingulata* (Stoneman) S. and von S., since the latter has been used similarly in numerous physiological and fungicidal studies. To his surprise, however, a supply of dextrose alone did not materially increase the percentage of germination of this fungus. After much experimentation, complete normal germination was finally obtained in a solution containing small quantities of dextrose, ammonium nitrate, mono-potassium phosphate and magnesium sulphate. The salts supplied evidently serve as nutrients. The following describes some experiments which lead to this conclusion.

MATERIAL AND METHODS.—The fungus culture supplied by Dr. C. T. Wei was originally isolated from plums. It produces an enormous number of spores in a short period of time. Sub-cultures were always made by smearing spores on potato-sucrose agar plates. Spores for germination experiments were obtained from the flooded surface of a three-day-old plate culture using a soft goat's hair brush (Chinese pen) to work them free. A spore suspension thus obtained is practically free from mixtures of conidiophores, mycelial bits and agar lumps. Removal of the chemical substances carried over from the original medium by the centrifugation method, and all other subsequent processes of the germination experiment by the petri dish method, were exactly the same as described in the previous paper

¹ Received for publication December 7, 1944.

Acknowledgment is made to Drs. L. M. Massey and L. Knudson of Cornell University for their keen interest in reading the manuscript, and thanks are due to Dr. C. T. Wei of Nanking University for the original fungus culture.

Published under a special ruling of the Editorial Board.

(1940). Readings of the percentages of germination were made 12 hours after the start of the experiment.

EXPERIMENTATION.—As in the case of some earlier investigators, the writer also observed that the cleaned spores which do not germinate in pure water germinate luxuriantly in a dilute plant decoction such as apple juice. After it was found that the supplement of dextrose only was ineffective in promoting germination, several experiments were carried out to test the activity of vitamins and auxins including thiamin chloride, nicotinic acid, ascorbic acid, glutathione, inositol and indoleacetic acid alone and in combination. The results were in all cases negative.

The first successful experiment was one in which a combination of three mineral salts was added besides dextrose (table 1).

The results clearly indicate that the addition of a single salt like magnesium sulphate, which always insures full germination of the conidia of *Sclerotinia fructicola*, does not act similarly in the case of *G. cingulata*. Nor was a solution containing two salts effective. The use of three salts containing essential nutrient elements, however, brought about full germination with robust germ tubes such as observed in a plant decoction.

It now seemed desirable to perform a routine test for the indispensability of various ions for germination, such as is done in a mineral nutrition experiment.

The results shown in table 2 indicate the indispensability of compounds containing nitrogen, phosphorus, magnesium and carbon, but not potassium and sulphur.

Further experiments were conducted to test the effect of potassium and sulphur in a solution in which

TABLE 1. *Effect of the composition of the medium on the germination of the conidia of Glomerella cingulata. Number of spores per cc., 17,000. Each figure represents the average of ten replicate counts on two petri dishes.*

Composition of the germination medium ^a	Per cent germination
None (re-distilled water)	0.9
Dextrose	8.1
Dextrose + MgSO ₄	18.1
Dextrose + KNO ₃	0.6
Dextrose + KH ₂ PO ₄	0.3
Dextrose + Ca(NO ₃) ₂	3.8
Dextrose + MgSO ₄ + KH ₂ PO ₄	40.4
Dextrose + KNO ₃ + Ca(NO ₃) ₂	5.1
Dextrose + MgSO ₄ + KNO ₃ + KH ₂ PO ₄	99.9
Dextrose + MgSO ₄ + KNO ₃ + KH ₂ PO ₄ + Ca(NO ₃) ₂	95.9

^a In all cases, the concentration of dextrose was 0.05 per cent, that of each of the mineral salts 1.0 millimol.

TABLE 2. *Essentiality of various ions for the germination of the conidia of Glomerella cingulata. Number of spores per cc., 24,000. Each figure represents the average of ten replicate counts on two petri dishes.*

Chemical substances supplied ^a	Element lacking	Per cent germination
None (re-distilled water).....	Carbon and minerals	0.0
Dextrose	Minerals	0.0
Dextrose + KNO ₃ + KH ₂ PO ₄ + MgSO ₄	None	80.4
Dextrose + NH ₄ Cl + KH ₂ PO ₄ + MgSO ₄	None	92.8
Dextrose + KCl + KH ₂ PO ₄ + MgSO ₄	Nitrogen	3.9
Dextrose + NaNO ₃ + NaH ₂ PO ₄ + MgSO ₄	Potassium	84.1
Dextrose + KNO ₃ + KCl + MgSO ₄	Phosphorus	1.5
Dextrose + KNO ₃ + KH ₂ PO ₄ + MgCl ₂	Sulphur	79.3
Dextrose + KNO ₃ + KH ₂ PO ₄ + Na ₂ SO ₄	Magnesium	0.9
KNO ₃ + KH ₂ PO ₄ + MgSO ₄	Carbon	0.7

^a In all cases, the concentration of dextrose is 0.01 per cent, that of each of the mineral salts 1.0 millimol.

the amounts of the other salts were much decreased in order to minimize the amounts of potassium and sulphur which might be present as impurities. As table 3 indicates, the requirement of sulphur is clearly demonstrated, but no striking difference in germination can be detected in potassium-containing and potassium-free media.

TABLE 3. *Effect of potassium and sulphur on the germination of the conidia of Glomerella cingulata in highly diluted media. Number of spores per cc., 15,000 in experiment 1, 44,000 in experiment 2. Each figure represents the average of twenty replicate counts in 4 petri dishes.*

Germination media ^a	Per cent germination	
	Experiment 1	Experiment 2
Full nutrient solution.....	80.0	70.9
Sulphur lacking	20.4	24.1
Potassium lacking	61.9	95.6

^a Concentration of dextrose 0.005 per cent, NH₄NO₃ 0.1 millimol, KH₂PO₄ 0.01 millimol, MgSO₄ 0.01 millimol, MgCl₂ 0.01 millimol, NaH₂PO₄ 0.01 millimol.

The determination of the minimum mineral requirement has been successful only in the case of nitrogen and phosphorus, which are presumably the major elements concerned in germination. The data presented in tables 4 and 5 indicate that approximately three gamma are sufficient to bring about full germination of thirty to forty thousand spores. The requirements of nitrogen and of phosphorus are of the same order of magnitude.

DISCUSSION.—Early investigators studying spore germination by the Van Tieghem cell method usually found no difficulty in the germination of conidia of *G. cingulata* in distilled water (Hawkins, 1913, p. 63). This was undoubtedly due to the carrying-over of sufficient nutrient material from the original medium by direct transfer. Goldsworthy and Green (1938) observed that most of the spores planted on water-agar blocks subjected to a 24-hour continuous flow of distilled water did not germinate. Those

spores planted on potato-dextrose-agar blocks, however, germinated rapidly. The authors assumed that

TABLE 4. *Nitrogen requirement in the germination of the conidia of Glomerella cingulata. Basic medium: dextrose 0.01 per cent, MgSO₄ 1.0 millimol, KH₂PO₄ 1 millimol. Number of spores per cc., 36,000. Each figure in column 3 represents the average of five replicate counts in each petri dish.*

Concentration of		Per cent germination
NH ₄ NO ₃ (millimol)	Nitrogen gamma/cc.	
None	None	0.0–2.5
0.001	0.028	13.6–15.7
0.002	0.056	41.7–50.6
0.004	0.112	80.2–84.0
0.01	0.28	96.3–96.4
0.02	0.56	98.8–99.3
0.04	1.12	99.9–99.9
0.1	2.8	98.8–99.2

TABLE 5. *Phosphorus requirement in the germination of the conidia of Glomerella cingulata. Basic medium: dextrose 0.01 per cent, NH₄NO₃ 0.2 millimol, KCl 0.1 millimol, MgSO₄ 0.1 millimol. Number of spores per cc., 30,000. Each figure in column 3 represents the average of 5 replicate counts in each petri dish.*

Concentration of		Per cent germination
NaH ₂ PO ₄ (millimol)	Phosphorus gamma/cc.	
None	None	0.5–1.6
0.001	0.031	7.7–10.9
0.002	0.062	28.4–37.5
0.004	0.124	65.8–78.4
0.01	0.31	91.7–98.3
0.02	0.62	94.8–96.7
0.04	1.24	98.2–99.5

the germination is made at the expense of some soluble accessory substance (1938, p. 494).

So far as the present investigation goes, it seems

certain that the conidia of *G. cingulata*, after being washed by centrifugation, demand an external supply of most of the major nutrient elements for germination. In case of *Sclerotinia fructicola* (Lin, 1940), the only material required for spore germination is a suitable source of carbon. Mineral salts which sometimes promote spore germination of *S. fructicola* play some physiological roles other than direct nutrition. Since several of the mineral elements found to be necessary for the germination of the conidia of *G. cingulata* are not replaceable, there is little doubt that in this case they function as nutrients.

Apparently the conidia of *G. cingulata* have so little reserve of the carbon source and other nutrients that they are unable even to initiate the earliest stage of growth. Whether there are nutrients in the gelatinous matrix of the spore mass sufficient for their use in germination is not known. In any case, it is quite possible that the germination of rapidly-formed spores such as the conidia of *G. cingulata* depends on some mineral nutrients as well as on energy material supplied by the new substratum.

SUMMARY

Normal germination of the cleaned conidia of *Glomerella cingulata* has been obtained by using a full nutrient solution composed of dextrose, magne-

sium sulphate, ammonium nitrate and potassium phosphate. The elements carbon, magnesium, nitrogen, phosphorus and sulphur have been proved to be indispensable, and the minimum requirement of nitrogen and phosphorus has been found to be of the order of 10^{-4} gamma per spore. It has not been possible to demonstrate the indispensability of potassium. There is no evidence that an external supply of any organic substances other than sugar is necessary.

This is believed to be the first report of mineral nutrition in the germination of fungus spores.

PLANT PATHOLOGY LABORATORY,
THE UNIVERSITY OF NANKING,
CHENG TU, CHINA

LITERATURE CITED

- GOLDSWORTHY, M. C., AND GREEN, E. L. 1938. Effect of low concentrations of copper on germination and growth of conidia of *Sclerotinia fructicola* and *Glomerella cingulata*. Jour. Agric. Res. 56:489-505.
- HAWKINS, LON A. 1913. The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. Physiol. Res. 1:57-92.
- LIN, C. K. 1940. Germination of the conidia of *Sclerotinia fructicola*, with special reference to the toxicity of copper. Cornell Univ. Agric. Exper. Sta. Mem. 233:1-33.

SOME ECOTYPIC RELATIONS OF DESCHAMPSIA CAESPITOSA¹

William E. Lawrence

THIS PAPER presents the results of an inquiry into the ecotypic relations of *Deschampsia caespitosa* (L.) Beauv. These have been assembled under two general topics: (1) the results of experiments on this species and (2) the significance of the ecotype concept in ecology.

Deschampsia caespitosa affords good material for the study of ecotypes. The variability of this grass indicates that its genetic diversity may correspond to the differences in ecological conditions obtaining throughout its wide geographic range. By bringing together individuals from geographically diverse regions to be grown in a uniform environment, a simple test is afforded of genetic diversity. Differences in the growth and behavior of these individuals reveal inherent genetic reactional characteristics of the various populations which they represent. The separate populations from each ecologically diverse region may constitute a genetically and physiologically distinct race complex called an ecotype. The existence of ecotypes in a number of species has been demonstrated by Turesson (1922a, 1922b, 1925), Stapledon (1928), Gregor (1930), Sinskaia (1931), Clausen, Keck, and Hiesey (1940), and others. It cannot be presumed, merely because ecotypes have been shown to exist in certain

species, that all taxonomic species are similarly constituted. Nor has any method been discovered by which they may be recognized in nature without experimental test, although their presence may often be suspected.

This paper presents evidence to show that ecotypes occur in *Deschampsia caespitosa*. It includes material, experiments, and records made available to the author through the Division of Plant Biology of the Carnegie Institution of Washington, at its central laboratory at Stanford University, California. The author wishes to express his grateful appreciation for this courtesy to Dr. H. A. Spoehr, Chairman of the Division, and to Drs. Jens Clausen, David D. Keck, and William M. Hiesey for their generosity and kindly assistance throughout the investigation, including the preparation of this paper. Garden records, herbarium specimens (vouchers), and fixed materials for cytological study were made available for this investigation. An opportunity was also afforded to study the experiments at the Stanford, Mather, and Timberline transplant stations. During the summers of 1942 and 1943 several weeks were spent in company with Dr. Clausen, and later with Dr. Hiesey, at the two mountain stations, where observations were made on the experiments and on the ecological conditions and floras of the environs.

¹ Received for publication December 27, 1944.

EXPERIMENTAL METHODS IN RACE ECOLOGY.—Wherever the relation of species to their natural surroundings is considered, two sets of variables are operative—the plant itself and the environment. It is difficult to separate them or to consider their component elements one by one. They cannot be critically differentiated when studies are limited to observations in the wild, nor is it possible to carry the whole of laboratory controlled technique into the field, yet fundamental studies on species in relation to their surroundings have recently been made by experimental methods. In this method one attempts to control the variables, so that only one factor is varied at a time. Such technique can be only approximated in field experiments. Two approaches have been employed with success—(1) the growing of individuals of different origin in a uniform garden as practiced by Turesson (1922a, 1922b) and (2) the growing of clone members of selected species in two or more ecologically separated uniform gardens, as advocated by Bonnier (1890), Hall (1926), and Clements (1929).

Perhaps nowhere have such experiments been carried out so well as in the mid-California transect of three climatic stations selected and established by H. M. Hall and associates and continued by Clausen, Keck, and Hiesey since 1932. These gardens at 100, 4,600 and 10,000 feet altitude, respectively, all have a relatively uniform soil and nearly level surface with open exposure. The plantings are kept free from weeds and contaminating seedlings, thus removing the factor of competition and the question of individual identity. Continued vigilance year after year is necessary in order to eradicate potential contaminants. It is hardly possible to appreciate the amount of labor and care that have already entered into the establishment and maintenance of these stations in order to meet the scientific requirements of field experimentation.

THE IMPORTANCE OF MODERN METHODS TO ECOLOGY AND AGRICULTURE.—Much of the comparatively recent ecological literature deals with the relationships of plant communities, their composition, distribution, and succession. This has led a part of the workers in other fields to regard synecology as the main content of ecology. At present there is developing a renewed interest in autecology, i.e., the nature of the individual and of the species, and of their relations with the environment.

Ecology is not primarily concerned with the discovery of the genetical mechanisms governing the origin and decline of species, nor with the nomenclatorial problems growing out of the diverse nature of populations as ends in themselves. An understanding of the relationships of organisms to their surroundings with reference to their fitness for survival, productive capacity, sociability, occurrence, and distribution is the real objective. From this point of view ecology contributes directly to biosystematics, as Clausen, Keck, and Hiesey (1945) designate this multiple approach to the study of evolutionary relationships. The solution of

these problems requires coordinated discoveries in genetics, cytology, ecology, taxonomy, and other branches of biology.

The integrating concepts of experimental taxonomy or biosystematics are producing a more comprehensive understanding of species and the subordinate population categories. These investigations, based in part on ecology, are gradually revealing the true relationships of species with the environment. Turesson (1922b, 1925) found by collecting samples of a species from contrasting geographic areas and growing them in a uniform environment, that they represented distinct climatic races, which he called ecotypes or climatic ecotypes. Tedin (1925), Gregor (1931, 1938) and Sinskaia (1931) have found evidence for the existence of biotic ecotypes. That other kinds of ecotypes exist is also indicated, such as edaphic ecotypes (Gregor, 1942). Clausen, Keck, and Hiesey (1940) demonstrated that the differences in climatic ecotypes are inherent in the plant and not altered by transplanting from one environment to another.

Morphological and physiological concepts of adaptations characterized the early approach to ecology. Most of this work has been based upon individuals from which generalizations have been made as though they were equally applicable to every race of the entire species. However, species are abstract units into which individuals are classified according to the author's particular concept. Moreover, species have been arbitrarily subdivided into subspecies, varieties, formae, races and strains. Therefore, classifications based wholly upon morphological characters often do not have biological significance. Biologists in general have frequently disregarded what the taxonomist already knew, i.e., that no one plant or group of plants represents the taxonomic species in its entirety. It is now well established that many species are reducible to smaller genetically and ecologically distinct units—the ecotypes.

One of the earlier attempts to explain the origin of species was based on the assumption that species differences were brought about by environmental factors acting upon individuals in such a manner as to change one species into another (Bonnier, 1890; Clements, 1929, 1937, 1939). It is true that external influences have a marked effect upon growth and reproduction. Physiological reactions and morphological parts are modified by the environmental factors. These alterations are called modifications, and they are non-transmittable to the offspring. Though this view is not as yet universally accepted (Clements, 1929, 1939), the evidence points overwhelmingly toward this conclusion (Clausen, Keck, and Hiesey, 1940). A clear differentiation should be made between the environmental effects, or modifications, upon the individual, and those changes that arise from one generation to another as a result of genetic variation. Experimental investigations on the nature of spe-

cies which do not keep these differences clear fail of their purpose.

From the point of view of plant indicators, the individual has been used as a representative of the entire species very largely without critical interpretation. Plant communities differ not only in their specific but also in their ecotypic composition. From our present knowledge it is evident that the ecotype, rather than the whole species, is the indicator. This is well illustrated by the geographic distribution of the ecotypes of *Deschampsia caespitosa* to be discussed below.

The ecotype concept aids our understanding of natural selection. It is clear that if species populations are composed of ecotype populations, the selection affects the more specialized group first. Species survival, therefore, depends upon ecotype survival.

In agriculture, where human standards form the basis of selection, the survival of plants depends upon the genetic fitness of strains and races to the particular combination of conditions involved in agricultural practice. Populations which fulfill these requirements are ecotypes in the same sense as applied to wild plants. They are, however, usually called commercial or cultivated varieties, or merely strains.

Range plants and timber trees are subject to the same kind of ecological selection as other plants in the wild or in cultivation. As they occur on the open range or in the forest they are ecologically fitted to the physical environment, and to their wild animal and plant associates. With the introduction of grazing by domestic animals and the cutting of the forest, an additional biotic selection under the influence of man is introduced. An examination of such areas may be expected to yield ecotypes of different kinds, and others in the process of formation.

Finally, it must be added that ecotypes can be identified and understood only through experimental study. These experiments, however, show that some ecotypes are morphologically recognizable. Once the ecotype investigations of any given species are completed, many other questions may be viewed and answered in the light of these results. Species migration, invasion, competition, and geographic distribution become problems involving ecotypes rather than entire species.

INVESTIGATIONS ON *DESCHAMPSIA CAESPITOSA*.—*Deschampsia* is a small genus of perhaps two dozen species, but only *D. caespitosa* (L.) Beauv. has been included in these experiments. This species has a primarily holarctic distribution, extending around the globe in the northern hemisphere but very rarely to the north of the Arctic Circle. At middle latitudes it is primarily a montane species, and it is almost absent from the tropics, even on the highest mountains, although it occurs in southern Mexico and is reported from parts of Africa. In the southern hemisphere forms referred to this spe-

cies occur in Australia, New Zealand, and Argentina.

A number of species have been proposed to cover the variation found within the *D. caespitosa* complex. The attempt is made here to clarify the situation only as it applies to western North America. For the purposes of these studies, all the material of this species and its relatives was studied in the Dudley Herbarium of Stanford University, the Herbarium of the University of California including the Vegetation Type Map Herbarium of the U. S. Forest Service and the T. T. McCabe collection on deposit there, and the herbaria of the California Academy of Sciences, Oregon State College, Willamette University, and the Carnegie Institution of Washington. Most of these collections were studied jointly with Dr. Clausen and Dr. Keck of the Carnegie Institution, so that the taxonomic conclusions represent the united opinion of the three workers.

The typical form of *Deschampsia caespitosa* as represented in northern Europe is marked by its open panicles, with small spikelets, glumes mostly 3–4.5 mm. long, and awns basally inserted, straight, and slightly shorter than the lemmas. This type occurs from Iceland across Eurasia from about the Arctic Circle southward to the Mediterranean and China. Westward it fails to occur in Greenland, but reoccurs in eastern North America from Newfoundland westerly to Pennsylvania, North Dakota, and Alberta. In western North America, in the area covered by the map, figure 1, this form occurs frequently, as indicated by the open circles, but even more commonly the awn is elongated, as shown by the solid circles, varying from a length just exceeding the lemma to one twice as long. These variations in awn-length show almost no correlation with geographic distribution, which precludes the possibility of successfully separating any part of the western North American material from genuine *caespitosa* by this character. The longer awns are more or less geniculate, but this character also appears to be of no aid in distinguishing natural units. The far western material tends to have larger spikelets than the far eastern and European material, but this character likewise varies, so that no morphological separation appears possible between European and West American *caespitosa genuina*.

Around the Gulf of Bothnia, at the head of the Baltic, a form occurs that differs from typical *caespitosa* only in having glumes 5–7 mm. long and awns much exceeding the lemmas. This is frequently called *D. bottnica* (Wahl.) Trin., but from the nature of the variation found within *caespitosa*, this appears to be but a subspecies of that, to which Wahlenberg originally referred it as a variety.

In the Alaskan area two variations are prominent. One is the common form of *genuina* as it occurs in western America. The other has much larger spikelets, the narrow glumes measuring 5–7 mm. long. In this form the awn is usually longer than the lemma, but sometimes it is as short as that in

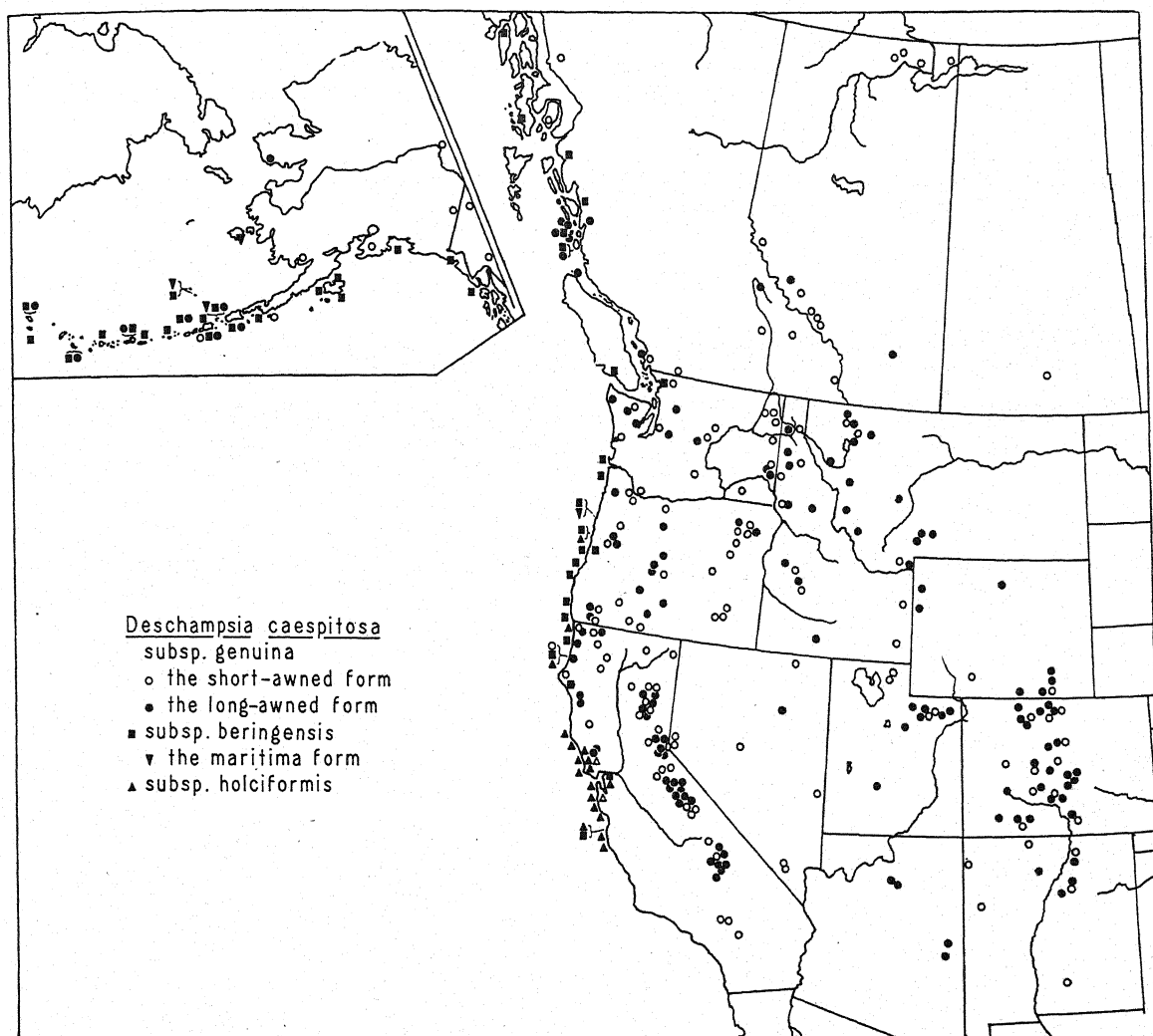


Fig. 1. Distribution of members of the *Deschampsia caespitosa* complex in western North America.

caespitosa genuina of Europe. This typically tall and robust form is found from Kamtchatka and the Aleutian chain, along the immediate coast of Alaska and British Columbia, southward to northern California (ssp. *beringensis*, fig. 1). Its panicle is open as in *genuina*, and it is the predominant form of the northern islands, although it is replaced by *genuina* toward the interior of the Yukon and southward. This large-glumed form has been named *D. beringensis* Hultén, but a complete series of intergrades connects it with *caespitosa genuina*. There is no likelihood that it is a distinct ecospecies, although it has the quality of a subspecies, to which category it is here referred. It can scarcely be distinguished from *D. bottnica*, mentioned above, but the geographic continuity of these forms has not been established, and it is believed that they have arisen independently. Related long-glumed forms are known from other coasts, however, as that of Labrador.

In the Aleutian Islands and along the northwest

coast *D. caespitosa* ssp. *beringensis* is typically a tall grass, frequently a meter or more in height. In the Pribilof Islands, however, and here and there elsewhere, it is found intergrading with an extra maritime form that attains a stature of only 15–25 cm. This type often has glumes less than 5 mm. long, as in the West American forms of *genuina*. It is found very scatteringly from the central coast of Oregon northward, and it is probably that which has received the name *D. caespitosa* var. *maritima* Vasey, described from Vancouver Island material 6–8 inches high. An Oregon form of this is now in cultivation, and it should be determined eventually whether or not this is subspecifically distinct. Its occurrence is indicated on the map (fig. 1).

Finally, along the California coast one finds a form that differs from *D. caespitosa* ssp. *beringensis* principally in its more condensed panicles, *D. caespitosa* ssp. *holciformis* (Presl) Lawr. This has been treated as a species ever since its proposal by Presl in 1830. The extreme form from Monterey,

California, at the southern end of the range of this unit, was the one originally described, but even that far south occasional plants are quite indistinguishable from ssp. *beringensis*, as indicated in figure 1. Along the central California coast the populations of this unit are often quite distinctive, with stout divaricate culms of medium height, heavy condensed panicles, long glumes, and awns often more adnate to the lemma than in the other units, although this character is variable in all of them. From Humboldt County northward to Oregon, however, *holciformis* and *beringensis* appear to recombine their characters freely. The northern limit of *holciformis* is on the central coast of Oregon.

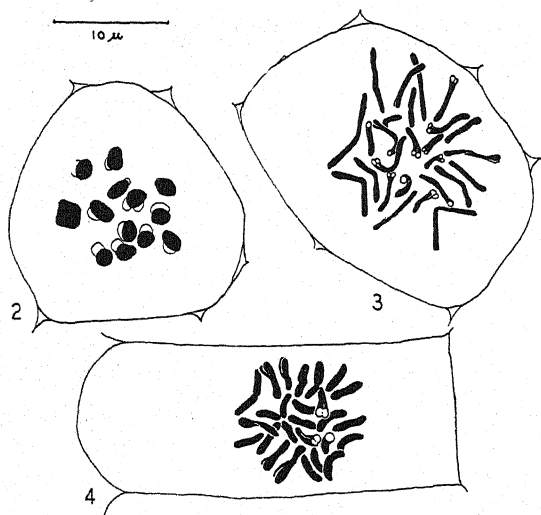


Fig. 2-4. Chromosome plates of *Deschampsia caespitosa*. Fig. 2 is a meiotic division ($n = 13n$) of a plant from Lapland, and figure 3 is a somatic division ($2n = 26$) of one from Tuolumne Meadows, at 8600 feet in the Sierra Nevada, both of which are referable to ssp. *genuina*. Fig. 4 is a somatic division ($2n = 26$) of a plant from Waldport, on the Oregon coast, referable to ssp. *beringensis*.

All forms of *Deschampsia caespitosa* are characteristically found in moist or wet places including bogs and in mountain meadows. Permanent ground moisture and a relatively cool to cold climate appear to be the limiting factors governing their distribution.

Key to the American subspecies of Deschampsia caespitosa

Panicle open at time of flowering, the branches drooping.

Glumes less than 5 mm. long; awn shorter than or equalling the lemma, except in western North America where it usually exceeds the lemma. 1a. *D. caespitosa* ssp. *genuina*

Glumes 5-7 mm. long; awn usually exceeding the lemma. Northwest coast. 1b. *D. caespitosa* ssp. *beringensis*

Panicle condensed at time of flowering; erect, glumes 4-7 mm. long; awn exceeding the lemma, frequently \pm adnate. California coast.

1c. *D. caespitosa* ssp. *holciformis*

1a. *DESCHAMPSIA caespitosa* subsp. *genuina* (Reichenb.) comb. nov. (*D. caespitosa* (L.) Beauv., Agrost. 91, 149, 160, pl. 18, 1812; based on *Aira caespitosa* L. *Aira caespitosa genuina* Reichenb., Ic. 1:pl. 96, fig. 1682, 1834. American synonymy detailed in Hitchcock's Manual of the Grasses of the United States 833-834, 1935). Densely tufted, with several erect culms 5-15 dm. high (on alpine peaks often considerably reduced), leaves principally basal, flat or folded, short or elongated; panicle open, nodding, the spikelets 5 mm. or less long, green to darkly anthocyanous; awn subbasally attached, rarely \pm adnate, usually shorter than the lemma in Europe and eastern America, and usually longer than the lemma in western America, straight or somewhat geniculate. In western North America occurring from central Alaska and the Yukon southward through the mountains to New Mexico, Arizona, and southern California, ascending to alpine peaks 12,500 feet high in Colorado and California, approaching the coast in places as far south as Humboldt County, California, but there mostly replaced by one of the following.

1b. *DESCHAMPSIA caespitosa* subsp. *beringensis* (Hult.) comb. nov. (*D. beringensis* Hultén, Fl. Kamchatka 1:107, 1927; type locality: Bering Island.) Differing from *genuina* in the long narrow glumes 5-7 mm. long, with the other floral parts (lemmas, anthers, and awns) correspondingly increased in size; awn usually much longer than the lemma and geniculate, but sometimes merely equaling it. Strictly coastal, extending from Kamchatka, the Commander and Pribilof Islands, along the Aleutian chain and the southern coast of Alaska southward to northern California. Quite uniform throughout except as to the maritime dwarf types previously mentioned, but intergrading with the other two subspecies.

1c. *DESCHAMPSIA caespitosa* subsp. *holciformis* (Presl) comb. nov. (*D. holciformis* Presl, Rel. Haenk. 1:251, 1830; type locality, Monterey.) Culms erect to divaricate, 5-12 dm. high; panicle condensed, the branches short, appressed, many-flowered; glumes 5-6 mm. long (occasionally only 3.5 mm.); awn exceeding lemma, usually \pm adnate. Rare on the Oregon coast; common from northern California to Big Sur, occasionally found somewhat inland as at Santa Rosa, hills back of Burlingame, etc. Intergrading particularly with ssp. *beringensis*.

Chromosome situation.—Chromosome counts have been made from root-tips and buds of individuals of *Deschampsia caespitosa* ssp. *genuina* from Lapland, southern Sweden, southern Finland, five localities from 6,200 to 10,000 feet elevation in the Sierra Nevada, and coastal Humboldt County, California. All had the same number of chromosomes, namely, $n = 13$, $2n = 26$ (fig. 2-4). The same number was found in individuals of ssp. *beringensis* from two localities on the Oregon coast and in the dwarf maritime form previously mentioned from exposed bluffs at Boiler Bay State Park, Oregon. The individual chromosomes vary in size. It

is obvious that the morphological and ecological variability found within this species is not connected with differences in chromosome number, but with the genetic diversity within the chromosomes.

The gametic number of 13 chromosomes is at variance with that reported by Hagerup (1939), namely, $n = 14$ in a form from Denmark, and $2n = 28$ in the *bottnica* form from Finland. Hagerup also reported $n = 14$ in the closely related *D. arctica* (Spreng.) Merr. (= *D. brevifolia* R. Br.) and in *D. pumila* (Ledeb.) Ostenf., but the shape of the chromosomes in his figure 4 indicates that the 14 chromosomes in *arctica* were counted in a somatic instead of a meiotic division. Accordingly, this far northern plant is diploid, with only 7 pairs of chromosomes.

Another close relative of *D. caespitosa* is *D. alpina* (L.) Roem. & Schult., which differs in being viviparous and in occupying an ecologic zone to the north of *caespitosa*. Hagerup found $2n = 56$ in a form of it from near Bergen, Norway, and Flovik (1938) reported $2n = 39, 41$, and 49 chromosomes in three biotypes from Spitzbergen. *D. alpina* is therefore approximately hexaploid to octoploid. The high polyploidy and variation in chromosome number observed in *alpina* is characteristic of species that propagate asexually.

D. caespitosa must be considered a tetraploid species, but its chromosome number ($n = 13$) is rare in its tribe of the Gramineae, which usually follows a 7-series. The number 13 suggests that *caespitosa* may have arisen from ancestors with 7 and 6 pairs of chromosomes.

SOURCES OF MATERIALS.—The individuals used in these experiments from Finland, Swedish Lapland, and southern Sweden were grown from seed collected by Dr. Jens Clausen in 1936, and those from California, with the exception of the Big Lagoon collection, which was grown from seed, were transplants taken by Drs. Clausen, Keck, and Hiesey, and Mr. Malcolm Nobs. All are referable to ssp. *genuina* and are listed below by culture number.

- 3301—Skärälid, Skåne, southern Sweden, at 56° N. Lat., in mixed deciduous forest of *Fagus sylvatica* L., *Quercus Robur* L., *Betula* and *Alnus*, along brook in deep basaltic ravine.
- 3302—Ruotsinkylä, north of Helsinki, southern Finland, at 60°10' N. Lat., in mixed lowland forest of *Betula pubescens* Ehrh. and *Picea Abies* (L.) Karst. (*P. excelsa* Link), along a ditch in sandy soil.
- 3303—South of Abisko, Lapland, Sweden, at 68° 20' N. Lat. and 1,300 feet altitude, along streams and in boggy places in forest of *Betula tortuosa* Ledeb. and at the northern limits of *Pinus silvestris* L.
- 3315-1 and -2—Slate Creek Valley (Timberline transplant station), Mono County, California, in the Sierra Nevada at 10,000 feet altitude, in an open wet meadow with alpine vegetation.
- 3315-11, -14, and -15—Tenaya Lake, Yosemite National Park, Mariposa County, California, at

8,200 feet altitude, on a meadow slope surrounded by *Pinus Murrayana* Balf.

3315-21, -22, -23, and -24—Yosemite Creek, Yosemite National Park, Mariposa County, at 7,200 feet altitude, in a dry bed of a creek under *Pinus Murrayana*.

3315-31, -32, and -33—East of Carnelian Bay, Lake Tahoe, California, at 6,225 feet altitude, on boggy edges of a pond.

3350—Big Lagoon, Humboldt County, California, at high tide level on the sandy shore, and also in clay mud.

Seedlings and transplants from these collections were first grown in the Stanford garden of the Carnegie Institution. From each of the seedling cultures a small number of individuals were immediately set apart for clonal division before any differences could be seen that would influence the selection. These and the transplants were eventually divided, and the resulting divisions, when strong enough, were placed in the climatic gardens at Stanford, Mather, and Timberline. Each individual was thus grown simultaneously in three environments. In some cases replantings were necessary on account of accidental death.

The Division of Plant Biology of the Carnegie Institution of Washington maintains three transplant stations in California at different altitudes and in contrasting climates. The environment of each of these is briefly characterized below, but for a fuller account of these stations the reader is referred to "Experimental studies on the nature of species, I" by Clausen, Keck, and Hiesey (1940).

Stanford Interstation Garden.—Elevation 30 m. (100 ft.). Located at the central laboratory of the Division on the Stanford University campus. The climate supports an oak-savanna association climax. For many species the conditions are favorable for growth almost the year around. Snows are absent, though frosts may occur from early November to the middle of March. With rare exceptions the rains are limited to the winter and spring seasons, and the summers and falls are dry so that irrigation is necessary at periodic intervals in order to maintain satisfactory growing conditions comparable to those at Mather and Timberline.

Mather Interstation Garden.—Elevation 1,400 m. (4,600 ft.). Located at Mather, Tuolumne County, on the west flank of the Sierra Nevada. The climate is indicated by the California black oak—ponderosa pine—incense cedar—manzanita climax association (*Quercus Kelloggii* Newb.—*Pinus ponderosa* Dougl.—*Libocedrus decurrens* Torr.—*Arctostaphylos* spp.). Spring begins about April 1, when most of the snow has melted, though frosts often continue until early June. The summers are mild and characterized by hot days and cool nights with no rain. Snows may be expected between October and April, with minor falls occurring into May. The garden is in a moist meadow, but irrigation is necessary at times, especially for young plants during the latter part of the summer.

Timberline Interstation Garden.—Elevation 3,050 m. (10,000 ft.). Located in Slate Creek Valley, Mono County, at the east base of Mt. Conness, just east of the crest of the Sierra Nevada. The climate is subalpine to alpine, the valley being surrounded by high ridges and peaks rising to 11,200 and 12,550 feet. Small glaciers occur on protected slopes of the highest peaks, while snow banks persist well past mid-summer. The garden is situated in an alpine meadow on the valley floor from which snows do not usually melt until the latter part of June, which leaves a growing season of three months or less. Winter snows may accumulate to a depth of 12 to 18 feet. Surrounding the garden meadow is a subalpine mixed association of Murray pine (*P. Murrayana* Balf.) on the south-facing slopes and of white-bark pine (*P. albicaulis* Engelm.) on the northern slopes and less exposed ridges.

These three gardens are sometimes referred to as climatic stations, or altitudinal transplant gardens. It is recognized that some difference in soil moisture, texture, and mineral content occur in the three gardens. The soil at Timberline is a youthful granitic loam of light texture, which in meadows is moist throughout the growing season. At Mather the soil is also a loose granitic loam that in summer becomes dry and hot near the surface but remains moist lower down. At Stanford the soil is a heavy clay loam that dries out and bakes during the summer unless irrigated and tilled. These different soils at the stations tend to reflect the climatic conditions under which they have been formed.

EXPERIMENTAL PROCEDURE.—These experiments have been planned and carried forward as a part of the investigations into the nature of species by Drs. Clausen, Keck, Hiesey, and Martin (1942). Annual records of them have been kept from 1939–1943 inclusive. Observations were made on the following characters: general vigor, including height of tallest stems, number of flowering stems, and cover-diameter of vegetative plant; date of first flowers; date of first ripe seed; frost resistance; disease injury; survival; and other descriptive characteristics such as presence of anthocyanin, and the occurrence of vegetative apogamy (vivipary).

In most cases the taking of first measurements was delayed from one to two years after transferring to the gardens. This allowed for establishment of the plant. Any measurement of growth may be utilized to indicate the inherited differences between individuals and races grown under uniform conditions, or to determine the effect of different environments on one individual through its clone members. In any one uniform environment, the behavior of individuals of one race forms a *race-pattern* (fig. 5 and 8). Similarly, the behavior of members of one clone in different climates (fig. 6 and 7) has been called a *reaction-pattern* (Clausen, Keck, and Hiesey, 1940). Ecotypes differ both in reaction-pattern and race-pattern.

Reaction- and race-patterns are derived from in-

dividual records of growth performance and stages of development completed annually. Fundamentally these are both expressions of the genetical and physiological capacity of an individual or a race in all forms of vegetative and reproductive growth.

SEEDLING POPULATIONS.—When very few individuals from a natural population are used as the basis for experimental deductions, the question arises as to the homogeneity of the population or as to the nature and extent of the variation within it. Accordingly, the transplant individuals were supplemented by seedling materials grown in the Stanford garden of the six populations from Big Lagoon, Yosemite Creek, Tenaya Lake, Finland, southern Sweden, and Lapland.

The Big Lagoon population showed outstanding uniformity. All of the 27 individuals flowered annually. The height of each was measured in 1940 and 1941, and it was greater than the height of the population in its native habitat. The number of individuals in each stem height-class is shown in figure 5. It will be seen that these curves closely resemble the normal biological curve which would be expected from data derived from much larger samples.

The populations from Finland and southern Sweden consisted of 35 individuals each. A comparison of their growth curves (fig. 5) shows that

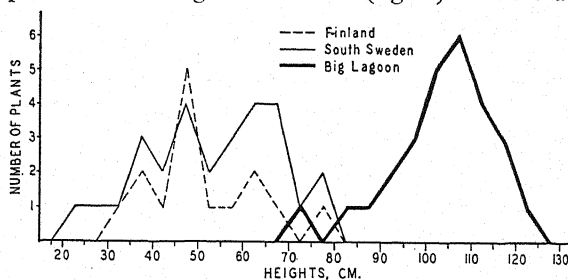


Fig. 5. Graphs illustrating frequency distribution of height in three seedling populations of *Deschampsia caespitosa* in a uniform garden at Stanford.

the tallest plants of the European races barely exceed the shortest of the Big Lagoon population. This indicates a genetic difference between these races. On the other hand, the genetic similarity in height of the two European races is shown by the close resemblance of their curves of variation for the two years. Of the 35 plants from southern Sweden, 25 flowered in 1938 and 28 in 1941. By 1941 four plants had died. Of the 35 individuals from Finland, 20 flowered in 1938 and 15 in 1941.

The population from Lapland consisted of ten plants, two of which were set aside for the transplant experiments. Of the remaining eight only two produced flower stalks, one of which was consistently prolific (viviparous). More than 31 degrees of latitude intervene between the home of this strain north of the Arctic Circle and the garden at Stanford. That plants from Lapland, Finland, and southern Sweden are able to grow and flower in a climate so very different is rather re-

TABLE 1. Height in cm. of culms in native habitats and at Stanford.

	Year	Race			
		Lapland	Southern Finland	Southern Sweden	Big Lagoon, California
In native habitat.....	1936	78.5	82	92.5	62
	1938	18 (1 plant)	42-80	22-74	87.5-127.5 (1940)
At Stanford	1941	31-42 (2 plants)	32.5-77.5	22.5-77.5	72.5-122.5

markable. It indicates a wide range of physiological tolerance and, when compared with the native Big Lagoon race, shows differences of an ecotypic character.

A comparison of the heights of the tallest flowering stems for the four populations is shown in table 1. The effect of cultivation and of the Stanford climate as compared with that of the native habitat has been to reduce the height of all the European races and to increase that of the Big Lagoon race. A comparison of vigor, height, floriferousness, and survival shows striking differences between races

from widely separated geographic areas having distinct climates. The two strains most alike are the ones from southern Sweden and Finland.

Sixty seedlings of the mid-altitude Sierran races from Yosemite Creek and Tenaya Lake were also set in the garden at Stanford, but they grew much more slowly than the other races, became attacked by rust, and soon died. Only 24 and 10, respectively, survived the first summer, after which the gaps were filled by new plants. In the following spring 21 and 15, respectively, were still alive, but only two of these flowered, and all died before the next

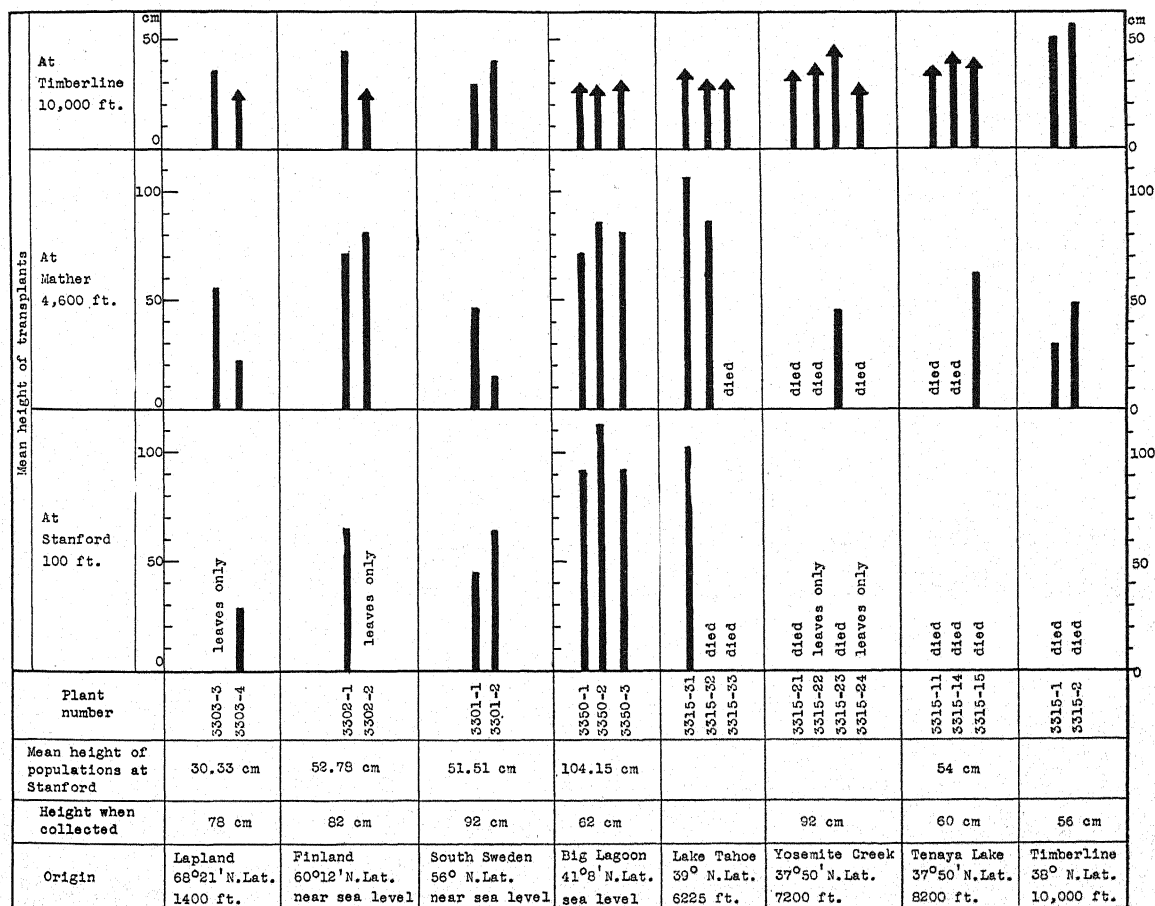


Fig. 6. Mean heights of plants of *Deschampsia caespitosa* at the three transplant stations. The arrowhead on certain columns at Timberline signifies that these individuals did not reach maturity any year.

spring. On the background of this poor survival record of the Sierran races, the performance of those from northern Europe is the more impressive.

TRANSPLANT EXPERIMENTS.—Two to four individuals from each habitat were divided and later transferred to the three climatic gardens. Being from one individual such divisions are genetically identical, so that any differences in the growth and behavior between them in the different environments represent phenotypic modifications. On the other hand, differences between individuals grown in the same environment represent genetic variation. These principles are basic to any experimental study of ecotypes. Height, date of first flowers, number of flowering stems, cover-diameter of the vegetative plant, and resistance to disease have been used as indices of ecotypic difference.

Height of tallest stems.—Figure 6 shows the mean heights of flowering stems for each individual at each station. The Big Lagoon race from the northern California coast is the most consistent in developing culms at all three altitudes. Its tallest stems are at Stanford, they are somewhat reduced at Mather, and they do not mature in the alpine climate at Timberline. The alpine race is taller at Timberline in its native environment, shorter at Mather, and it fails to survive at Stanford. The mid-Sierran races from Lake Tahoe, Yosemite Creek, and Tenaya Lake do not reach maturity at Timberline and survive poorly at the other stations, especially at Stanford, but they tend to be tallest at Mather. The Lapland, Finland, and South Sweden races survive at all three stations, but they are not very floriferous at Stanford, which makes measurement of stem height uncertain there. They tend to be tallest at Mather, with Stanford next, and only in the most favorable years do their stems attain full development at Timberline. The Lapland race is slightly shorter than the others. The short stem at Mather of 3301-2 from southern Sweden is probably accidental, for this plant did not establish itself there but died the second year and was never reset.

Using height as a criterion one finds striking differences in behavior in these geographic races. This is accentuated when one considers the reactions of each race in all three climates. But even at one station race differences stand out. At Stanford the Big Lagoon race is distinctly the tallest, at Mather the Lake Tahoe race is slightly the tallest, and at Timberline the native race from there excels the others. Each of these races is the one that in the altitude of its native habitat most closely corresponds to that of the station where it surpasses the others.

Number of flowering stems.—Different environments affect very profoundly the number of flowering stems per plant. The Big Lagoon race has many more stems at Stanford than at the other stations. The European races tend to have most stems at Mather, and the Timberline race is most floriferous at the alpine station, where the number of stems

has increased from year to year. Apparently any measurement of growth, as shown by these experiments, leads to similar results; for the data show that a correlation exists between the number of stems, their height, and the cover-diameter of the vegetative plant.

Cover-diameter.—This refers to the greatest diameter of the vegetative plant and is a partial expression of the vegetative shoot- and leaf-length. Cover as an ecological index is usually expressed in area (Braun-Blanquet, 1932), which is a function of the diameter. Cover has been applied chiefly to the role of species in the community.

Measurements of cover-diameter show, as did the number of flowering stems and their heights, that the Big Lagoon race is consistently largest at Stanford and smallest at Timberline, as one would expect. Likewise as to these measurements the European races are usually largest at Mather and second best in the Stanford environment. The Lake Tahoe race had approximately the same cover-diameter at Stanford and Mather, but was much reduced at Timberline. However, this plant will survive at Timberline and not at Stanford. These observations show that when geographic races are brought together and grown under uniform conditions they exhibit distinct physiological characteristics, which are based on genetic differences. Using cover-diameter as a criterion, no two geographic races show the same reaction-pattern.

Growth correlations.—A similarity in the growth patterns of individuals of *Deschampsia caespitosa* is revealed by measurements of (a) height of tallest stems, (b) number of flowering stems, and (c) cover-diameter of the vegetative plant. All of these are growth expressions of vigor. If the vigor of a plant is a characteristic of the whole organism, it may be expected to affect its parts proportionally. These experiments show that the growth patterns are similar, although not identical, for all three methods of measurement. For instance, plant 3350-1 from Big Lagoon produced the tallest stems at Stanford, the intermediate at Mather, and the shortest at Timberline, and the same reaction-pattern was found when growth was measured by number of flowering stems and by cover-diameter. Nearly all the individuals in the experiment would afford equally good examples of this correlation.

Vigor.—Certain quantitative methods of measuring vigor have been presented above. It is significant to add that the subjective estimates of vigor made by different persons in different years and seasons at the three gardens show a remarkable agreement with the quantitative measurements. Vigor was estimated by recording the plant as barely alive, weak, fairly strong, strong, very strong, vigorous, and very vigorous. At Stanford the Big Lagoon race is the most vigorous while the Sierran races are least so. The Big Lagoon plants (fig. 7) show a consistent reduction in vigor from low to high altitude while, in contrast, the

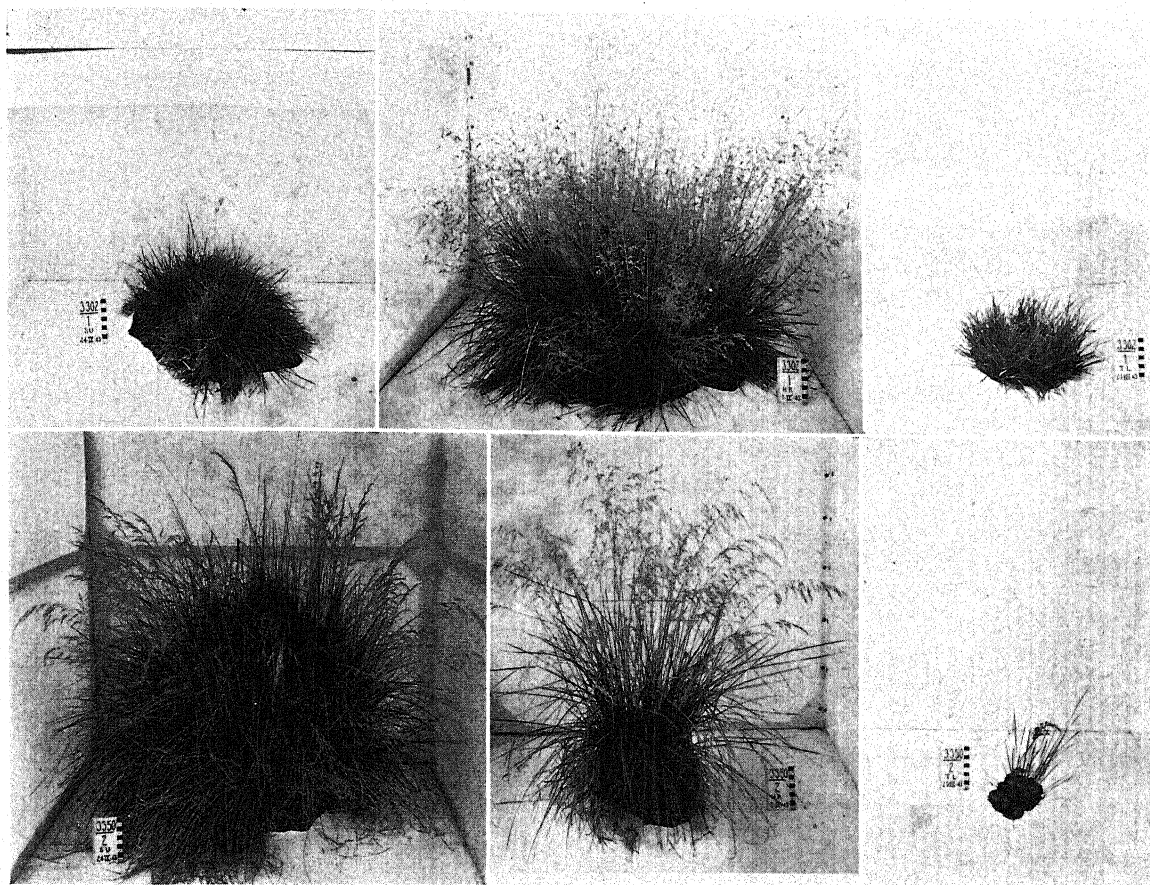


Fig. 7. Modifications at three altitudes in a clone of the Finland race (upper) and in a clone of the Big Lagoon race from the California coast (lower).

Timberline race shows an increase. The other Sierran forms, though generally weak at lower altitudes, are intermediate, for the Lake Tahoe race is most vigorous at mid-altitude, whereas those from Yosemite Creek and Tenaya Lake are strongest and most consistent in behavior at Timberline, although they are less vigorous there than

the native race. The northern European races are distinctly strongest at Mather (fig. 7).

Considering the racial differences as shown at any one station, we find that at Timberline, among the races from California, the plants from high altitude are the most vigorous, the vigor decreasing with the original altitude of the races (fig. 8).

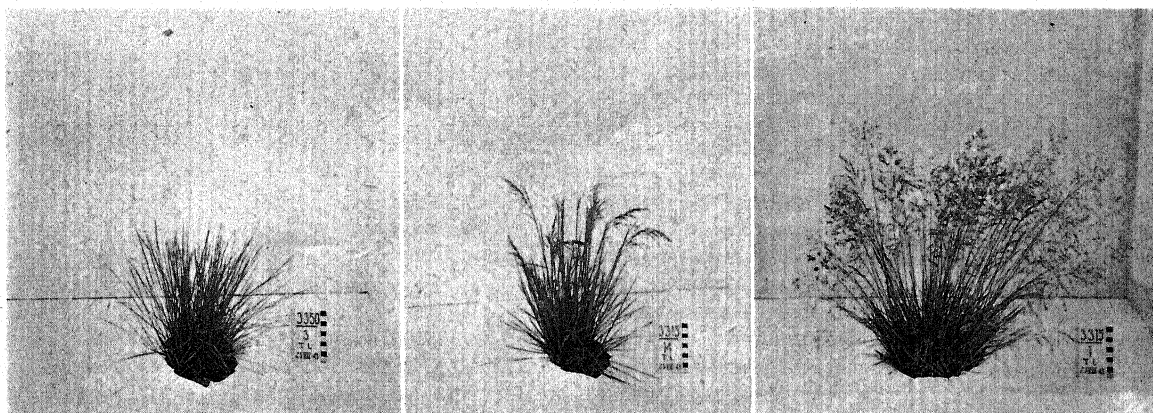


Fig. 8. Racial differences at one altitude as shown by plants from Big Lagoon (sea level), Tenaya Lake (8200 feet), and Timberline (10,000 feet), respectively, growing at Timberline.

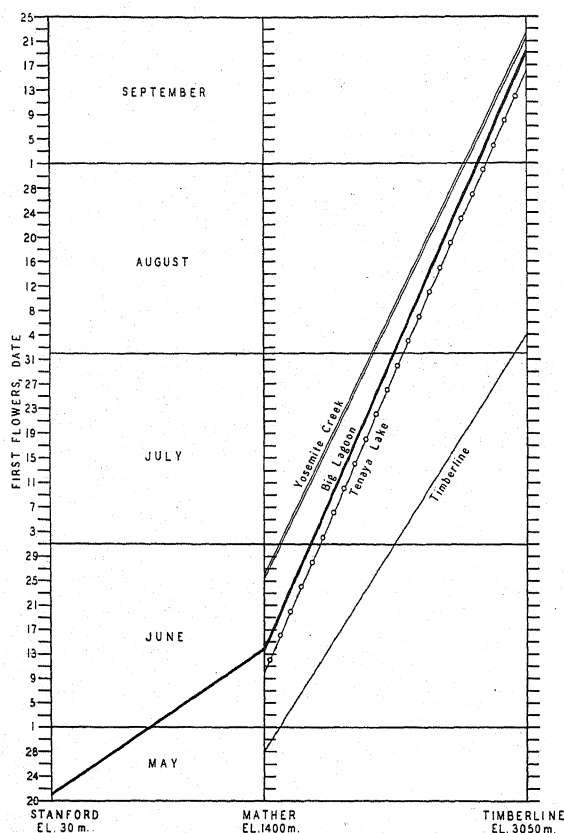


Fig. 9. Modifications in date of first flowers in clones of California races of *Deschampsia caespitosa* grown at three altitudes.

In contrast, the same plants at Stanford show a decrease in vigor following the increase in altitude of their original habitats. For these reasons it may be concluded that vigor, as measured by the standards above, is a genetic characteristic of geographic and climatic races; that it is subject to environmental modification; and that races differ in their patterns of modification.

Earliness.—Similarly, earliness is a genetic characteristic subject to environmental modification. It is important for survival in climates with short growing seasons. This could easily be tested in the three critical environments of the climatic gardens. The growing season at Stanford is terminated by drought and high transpiration, and at Timberline by early frosts. That the length of growing season is a critical factor at Timberline is shown by the ripening of seeds in favorable years on individuals that ordinarily fail to do so.

Figures 9 and 10 show the mean dates of first flowers for the 21 individuals at each climatic garden. Considering the reactions of an individual race, it will be noted that in every case the date of first flowers is delayed with rising altitude of the environment in which the plant is grown, except for the Lapland race, which flowers erratically at Stanford. The Timberline race is the

earliest at the mountain stations and at Timberline it flowers approximately one month ahead of any other, maturing seed almost every year (see fig. 11). The other races differ only slightly in earliness at Timberline, usually not flowering before mid-September or later. The subalpine forms from Tenaya Lake and Yosemite Creek are consistently later than the Timberline race, but they are slightly earlier than the transplants from Lake Tahoe. The coastal race from Big Lagoon is very consistent at all three stations in its time of flowering and, at Mather and Timberline, corresponds to the earliest subalpines.

In the European races there is a great spread at Mather, with the subarctic form from Lapland two to three weeks earlier than any other and comparable with the Timberline race. It is also earlier than the others at Timberline, but insufficiently so to permit it to ripen seed there in other than the most favorable years.

Floriferousness.—Figure 11 is a graphic presentation of the record of flowering and survival for each of the 21 individuals included in these experiments. Failure to flower characterizes certain individuals and races at Stanford and is supposedly an indication of lack of harmony between the plant and the climate. The only race uniform-

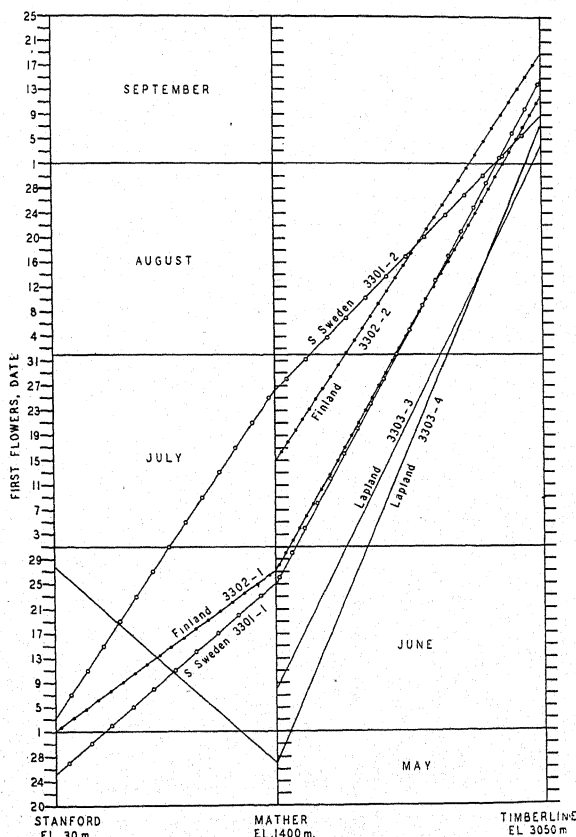


Fig. 10. Modifications in date of first flowers in clones of European races of *D. caespitosa* grown at three altitudes.

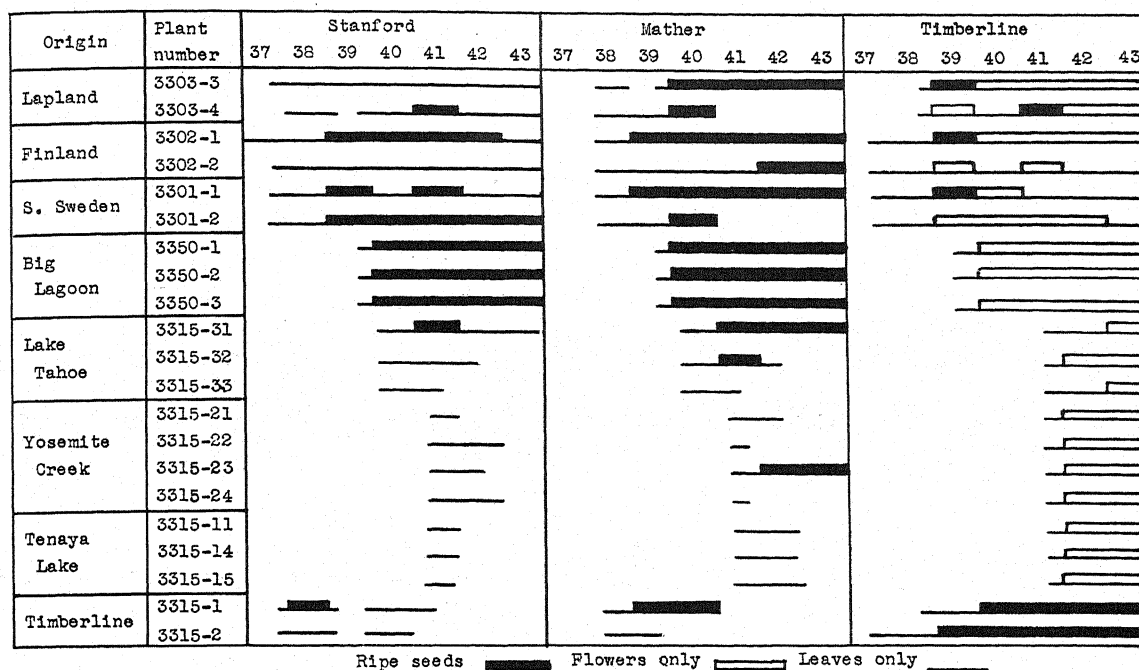


Fig. 11. Record of survival and flowering of clones of *D. caespitosa* at three altitudes.

ly floriferous at all stations is the one from Big Lagoon, for it flowered regularly even at Timberline, although it is not known to have produced ripe seed there.

The performance of the European races at Stanford is very erratic as compared with those from California. One individual of the Lapland race and one from Finland never flowered at Stanford. In fact, only three of the ten original seedlings from Lapland ever flowered at Stanford, and this Finnish plant bloomed only in the fourth and fifth years at Mather, and in the second and fourth years at Timberline. Performance of the Lapland individuals was best at Mather, although one died after two seasons, but both matured seed in favorable years at Timberline. Both plants from southern Sweden and one from Finland produced ripe seed at all three stations. Other experiments with inter-continental transplants substantiate the fact that individuals far removed climatically from their native habitat are more erratic in their flowering than forms grown in or near their original climate. The erratic flowering of the European *Deschampsias* is apparently due to individual genetic differences, which may not come to expression in their native environment, but are very important in the radically different California lowland climate.

Survival and vitality.—Survival of the individual and of the race depends upon the sum total of all inherent characters which determine fitness to the environment throughout the period of vegetative establishment and fruitfulness. Earliness, floriferousness, and susceptibility to disease and frost are but a few of the characters affecting survival. Here

survival is measured by the time interval during which an individual persists in a given environment. It is distinct from vitality as used by Braun-Blanquet (1932). Vitality is the capacity of the organism for a certain degree of continued growth and to tolerate adverse conditions through a period of time.

The following stages of development attained by the end of the growing season have been used as indicators of the degree of vitality: (a) vegetative growth only, (b) flowers or unripe seeds, and (c) ripe seeds. The data are graphically presented in figure 11.

Considering first the native California forms, the Big Lagoon race has a remarkable survival record for a plant from the immediate coast, for it survives and flowers at all stations. At Timberline, all three individuals of it have survived for five years, but they have been unable to mature seed there on account of the short season. In previous transplant experiments, no other species from the coast has been able to survive in the alpine environment so long. This race thrives best, however, at the lowland station.

The races native to the Sierra Nevada survive well at the alpine station only, but the Timberline race is the only one able to produce ripe seed there every year. They have a high death rate at Stanford and Mather. We find, therefore, that these races survive best in the garden with a climate nearest to that of their own habitats.

The European races have a great range of tolerance, for they survive fairly well at all three stations. They are, however, not very well adapted to the California climates, as shown by their reduced

flowering, and at Timberline they ripen seed only in the most favorable years. Their best performance is at Mather, the climate of which most closely corresponds to that of their homes.

The degree of vitality and survival of an organism are expressions of the heredity of the plant. These are useful criteria of ecotypes, directly observable when the plants are grown in a uniform environment. In different environments these inherent characteristics are modified. Natural selection operates upon them, resulting in geographic and ecologic races. Sometimes these are correlated with recognizable morphological characters, or again, as in *Deschampsia caespitosa*, it is impossible to distinguish the ecotypes by morphology. For this reason, ecotype populations in this genus must remain for the present as experimental categories only.

Frost injury.—All the races of *Deschampsia caespitosa* investigated are very frost hardy. Only the individuals from Big Lagoon are slightly susceptible, but even this coastal race showed very little injury after severe frosts at 10,000 feet altitude. The frost hardiness of this species enables some of its races to mature seed at Timberline even when they flower fairly late.

Rust injury.—When grown at Stanford, all individuals from the Sierra Nevada die from the effects of plant rust disease, but all the European races and the one from Big Lagoon are resistant to it and survive there. When grown at Mather and Timberline, the Sierran races are free from rust. This has led to the suggestion that the problem of resistance to disease may be closely allied to the fitness of the plant for the climate in which it is being grown (Clausen, Keck, Hiesey, and Martin, 1942).

Other characteristics.—Some observations are available on the presence of anthocyanin and vegetative apomixis. In 19 of the 21 individuals in these experiments anthocyanin was present, varying from only a trace of purple color to dark purple. There is a general tendency for an increase in intensity of pigment when the plants are grown at high altitude, and even the greenish forms become purple at Timberline.

Vegetative apomixis, a term proposed by Stebbins (1941), has been called vivipary by many writers, especially in Europe. The same phenomenon is also referred to as proliferation. It is not of common occurrence among native grasses in the United States, though instances have been reported. It is more common in arctic and subarctic regions, and in northern alpine situations. Certain genera, such as *Festuca*, *Poa*, and others, are well known vegetatively apomitic grasses. *Deschampsia alpina* (L.) R. & S., as previously mentioned, is exclusively viviparous and marked by a much higher chromosome number than *D. caespitosa*, which does not occur so far north. *Deschampsia caespitosa* is generally sexual even in northern climates, although Hitchcock (1935) states that it is occasionally proliferous. An instance of vegetative apomixis was

accidentally produced by Jenkin (1922), when *caespitosa* was grown under abnormal greenhouse conditions.

The seeds from Lapland, Finland, and southern Sweden were secured from sexual populations. Nevertheless, when grown at Mather and Stanford, all six European transplant individuals have been viviparous, especially towards the end of the season. In certain plants almost all the florets developed into green bulblets that spectacularly weighed down the panicles. The degree of proliferation varied somewhat from year to year, but 3301-1, 3302-1, and 3303-4 were always viviparous to a much higher degree than the others. Under the alpine conditions at Timberline a trace of vivipary was found in 3303-4 in one year, but the others were purely sexual there. At this high altitude the season is probably too short for this character to express itself. None of the races from California have shown this tendency except the alpine one, which one year in the late fall developed a few bulblets in a greenhouse at Stanford. Since none of the races was viviparous in Europe, and only some became distinctly so in California, it is concluded that this form of vegetative apomixis must be produced by the interaction of heredity and environment.

Bulblets obtained at Mather from the very viviparous Finnish plant, 3302-1, developed into new plants. These had the same chromosome number as their parent, so vegetative apomixis in *D. caespitosa* is not accompanied by an increase in chromosome number to make this conspecific with its arctic relative, *D. alpina*.

ECOTYPES OF DESCHAMPSIA CAESPITOSA.—As previously stated, this study is based upon collections from eight localities in five distinct climates: subarctic in Lapland; continental cool temperate in Finland and southern Sweden; maritime temperate along the coast of northern California; continental montane, and continental alpine in the central Sierra Nevada. Even in a species so widely distributed as this one, the local races utilized in these experiments are unusually representative. They have been tested in three distinct climates: continental-lowland at Stanford, montane at Mather, and alpine at Timberline. From the results of these investigations at least five climatic ecotypes appear to be represented, and in the species as a whole more are to be expected. Except for an untested rare form from dry hillsides in Denmark, no data are available on the existence of edaphic ecotypes in this species.

These five climatic ecotypes are referred to as (1) a coastal ecotype of western North America, represented by the Big Lagoon race; (2) an upper (cool) montane or subalpine Sierran ecotype, represented by the Tenaya Lake, Yosemite Creek, and possibly the Lake Tahoe races; (3) an alpine Sierran ecotype, represented by the Timberline race from Slate Creek Valley; (4) a cold temperate ecotype of northern Europe, represented by the races

from Finland and southern Sweden; and (5) a subarctic ecotype, represented by the Lapland race.

1. *Coastal ecotype of western North America*.—The Big Lagoon race, which represents this ecotype, came from the Humid-Transition life-zone, which in northern California is characterized by the *Sequoia sempervirens*—*Pseudotsuga taxifolia* association. This race grows tallest and develops most flowering stems at Stanford, the climate of which most nearly approaches that to which it is native. It survives and flowers at all three stations, but has been unable to mature seed at Timberline. It has become gradually weakened during the five years it has persisted at this high elevation, where it is the only race at all frost-susceptible.

2. *Upper montane Sierran ecotype*.—This is tentatively recognized as including the Tenaya Lake (8,200 ft. altitude) and Yosemite Creek (7,200 ft.) races, and possibly the one from Lake Tahoe (6,225 ft.). The three were collected in the Canadian and Hudsonian life-zones in close proximity to *Pinus Murrayana* Balf., a species that occurs in both zones, although an altitude difference of 2,000 feet in this area presents changes in plant community composition. That these three races of *Deschampsia* show only slight differences in reaction-pattern may be due to the relatively narrow range of soil moisture tolerance in this species. The close association with *Pinus Murrayana* as a uniting species may indicate a similarity in the habitat.

The upper montane Sierran ecotype develops very poorly at Stanford, where all individuals become attacked by rust and die, and it scarcely survives better at Mather. At the alpine station, however, all of its individuals succeed and flower, although they usually flower too late for development of seed.

There are apparent differences between the races in this ecotype, for the Lake Tahoe plants are weaker at Timberline and slightly stronger at Mather than the others. Nevertheless, ecotypes are made up of smaller population units. Sinskaia (1931) refers to these as ecoelements, or elementary ecotypes, though it is a question whether additional terminology for populations within the ecotype can serve any practical purpose in field ecology.

3. *Alpine Sierran ecotype*.—The Timberline race, which represents this ecotype, came from the Alpine life-zone at the upper boundary of the Hudsonian in the high Sierra Nevada and occurs characteristically in the alpine grassland surrounding the Timberline transplant garden.

This ecotype resembles the upper montane Sierran one in most of its characteristics, except that it flowers much earlier. At Timberline it is approximately one month earlier than the earliest of the upper montane races. At Mather it is also earlier, although only by two or three weeks. At the alpine station its flowering stems are the tallest of all ecotypes (fig. 6), although they come from plants with a smaller cover-diameter.

The Timberline and Big Lagoon races are ecotypically opposites. Their original habitats are in alpine and maritime climates, respectively, with an altitude difference of 10,000 feet. Their reaction-patterns are directly reversed, for the Timberline individuals fail completely at Stanford, eventually die at Mather, but survive indefinitely and are very vigorous in their native alpine environment.

4. *Cold temperate ecotype of northern Europe*, represented by the Finland and southern Sweden races. The seeds of both of these races came from the mixed northern European forest. The southern Sweden form was collected in a beech wood in the northern part of the range of *Fagus sylvatica* L. The Finnish form was collected in a mixed forest of Norway spruce (*Picea Abies* (L.) Karst.) and the moor birch (*Betula pubescens* Ehrh.).

All the races from northern Europe, including the one from Lapland, develop most vigorously at the mid-altitude station at Mather, unlike any California race. The populations from southern Sweden and Finland show no conspicuous differences in reactions. The minor differences noted are not surprising in the light of their geographic separation. They are only moderately floriferous at Stanford, more so at the mountain stations, but they are usually unable to mature seed at Timberline. In earliness they compare with the upper montane Sierran races, but unlike them they are resistant to rust and survive at Mather and Stanford.

5. *Subarctic ecotype*, as represented by the Lapland race. This race came from north of the Arctic Circle near the border of the subarctic and arctic climates. It was associated with the mountain birch, *Betula tortuosa* Ledeb., at the northern and altitudinal limit of the coniferous forest in Swedish Lapland.

At the mid-altitude station this race is almost as early as the alpine Sierran one, and its first flowers appear there from three to six weeks earlier than those of the other European races. At Timberline, however, it is only slightly earlier than the others and seldom able to mature its seed. At Stanford it differs from the other European races by usually remaining in the vegetative stage and not flowering at all. Only once did it flower there and then very late in the season. This race stands out as a distinct latitudinal ecotype. In its fitness for survival it is less specialized than the alpine Sierran ecotype, the native habitat of which has a growing season considerably shorter than that of Lapland.

Morphologically, all of the ecotypes listed above belong to *D. caespitosa* ssp. *genuina*, but ecologically they represent units as diverse as the climates they occupy and the species with which they associate. Here is one subspecies that has developed races able to grow in climates as different as those of the mild California coastal zone near the redwoods, the high alpine grasslands of the Sierra Nevada, and the subarctic forests of Lapland. A species of such wide tolerance is not a good ecologic indicator, but its ecotypes are. The basic ecologic

unit is therefore not the taxonomic species or subspecies but the ecotype.

The ecologic effects of high latitude and of high elevations at lower latitudes are often assumed to be more or less similar. This has been supported by the fact that circumpolar and arctic species often extend far south in the high mountains. In the environs of the Timberline station are found, for example, such characteristic far northern species as *Phleum alpinum* L., *Trisetum spicatum* (L.) Richt., *Luzula spicata* (L.) DC., *Oxyria digyna* (L.) Hill, *Rhodiola rosea* L., *Sibbaldia procumbens* L., and *Veronica alpina* L. It is therefore worth noting that the alpine Sierran and the subarctic Lapland races of *Deschampsia caespitosa*, which in their native environments grow near these species, react very differently and so belong to distinct ecotypes that are not closely related ecologically. This should have been expected, because the Sierran alpine climate has a considerably shorter summer and also a much shorter day during the growing period than the Lapland climate. For these reasons it may be expected that species like those mentioned above also have ecotypes in the Sierra Nevada distinct from those in the far north.

NATURE OF ECOTYPES.—Ecotypes are race complexes of one species that are fitted to live in an environmental zone or niche. The real nature of ecotypes is undefinable, because as yet we do not know how any one of them has evolved. The fitness of an ecotype to its natural environment, however, appears to depend upon the interplay between complexes of hereditary and environmental factors, rather than upon any single factor. Depending upon the ecological factors primarily responsible for their development, different kinds of ecotypes arise.

Furthermore, the multitudinous relationships of an ecotype are not fixed but are continually changing, and we are faced with organic evolution taking place in an ever-changing environment. The ecotypes represent the final stage of adaptive evolution within the species, if we define the species as populations, the individuals of which can interbreed freely. Sensing this complexity in relationships, we seek simplification through classification of ecotypes and other biological units, only to discover that thereby we have multiplied the complexity.

Fundamentally the ecotype is not a taxonomic unit. This is perfectly illustrated by the situation in *Deschampsia caespitosa*, for the evolution and distribution of its taxonomic variants appear to be entirely independent of any ecotypic adaptation. Although a careful study of the distribution of the morphologic variants might have suggested such lack of relation between morphologic and ecotypic characteristics, it could never have been proved except by growing the plants in a uniform environment or in sets of such environments. Any correlations between this kind of morphological character and the physiological are largely incidental, depending upon gene linkage.

Ecotypes of one species are often contiguous in distribution, and a series of such ecotypes may replace one another over a given area. They are capable of free interbreeding, and hybrid recombinations may therefore be found where adjacent ecotypes meet. Some infusion of genes from neighboring ecotypes unquestionably takes place, but at some distance from the zone of contact the majority of the individuals in the local populations remain true to the ecotype of that area. It is apparent that it is the fitness of an ecotype to a particular environment that keeps it in place and, through natural selection, controls the spread of it and its hybrids.

The selective agencies should therefore determine any natural classification of ecotypes. Unfortunately but little is known about the operation of these determining factors. Theoretically there could be as many kinds of ecotypes as there are ecological factors operating in natural selection. Four general kinds of ecotypes have already been recognized and more or less supported by confirming experiments:

1. Climatic ecotype (Turesson, 1925)
Synonym: climatype (Sinskaia, 1928, 1931)
2. Edaphic ecotype (Sinskaia, 1928; Gregor, 1942)
3. Biotic ecotype (Sinskaia, 1931)
 - a. Synecotype (Sinskaia, 1931)
 - b. Agroecotype (Gregor, 1938)
4. Geographic ecotype
Synonyms: seclusion type (Turesson, 1927)
geocotype (Gregor, 1931)

The existence of climatic and biotic ecotypes is well established through many studies. The edaphically separated forms are often closely related ecotypes, rather than ecotypes, that is, they have internal, partial barriers to interbreeding in addition to those imposed by the environment. Although less common than the others, a few apparently good examples of edaphic ecotypes are on record.

The geographic ecotypes or seclusion types owe their distinctness to physical barriers against the free migration of genes, or to peculiarities in the distribution of genes. In this respect they are related to the geographical subspecies based on morphology. In cases where such units are correlated with climate or soils, they belong with the climatic or edaphic categories. If no special correlation to the environment exists, it is a question whether forms separated by mere distance constitute ecotypes in the real sense of the word.

IMPORTANCE OF THE ECOTYPE CONCEPT IN ECOLOGY.—During the past two decades evidence has been accumulating to show that the species is not the basic population unit most definitely related to the environment. The ecotype and not the whole species is the basic ecologic unit. The discovery that species occupying more than one major environmental zone, or niche, are composed of ecotypes has brought to emphatic realization the ecologically heterogeneous nature of the morphological or taxonomic species. The survival of *Deschampsia caespitosa* in any one of the environments it occupies depends not upon the length of its awns or glumes

but upon its physiological fitness to that environment, and this fitness is part of its inheritance.

The ecologic and taxonomic analyses of *D. caespitosa* have strikingly shown the difference between the morphological and the ecological concepts, for one kind of unit is superimposed upon the other. Five ecotypes, all belonging to ssp. *genuina*, have been recognized here, and unquestionably quite a few more could be discovered within other climatic zones covered by that subspecies. Conversely, it is just as probable that additional investigations would reveal that the California coastal ecotype includes not only those rare forms along the coast classified as ssp. *genuina*, but also the common ones referred to ssp. *holciformis* and *beringensis*, for even members of different genera native to this climatic zone tend to have similar ecological reaction-patterns (Clausen, Keck, and Hiesey, 1940). From this it is not to be construed that members of the different genera, or ecospecies, belong to the same ecotype.

It is therefore worthy of emphasis that the concept of ecotype must be completely divorced from the morphological concept of species, subspecies, or other taxonomic categories. The ecotype relates to natural population complexes of the same ecospecies fitted to the environment regardless of any morphological characteristics. The non-experimental subspecific taxonomic categories are more or less arbitrarily recognized assemblages of individuals having similar morphological characteristics in common. In some instances, however, ecological and morphological characteristics may be closely related, as in the subspecies of *Potentilla glandulosa* Lindl. (Clausen, Keck, and Hiesey, 1940).

In some instances a species consists of only one ecotype, in which case the ecotype is synonymous with the taxonomic species. Such monotypic species occupy only one major environmental zone. In other cases experiments have demonstrated that the species consists of many ecotypes, as in *Deschampsia caespitosa*. Such species have a wide distribution over many zones. If the actual relationships of organisms to their essential surroundings are to be interpreted correctly, the ecologist, the systematist, and the agriculturist must deal with the basic ecological unit, the ecotype.

SUMMARY

A study was made of 21 individuals representing eight races of *Deschampsia caespitosa*, clonal divisions of which were transplanted to the experiment stations of the Division of Plant Biology of the Carnegie Institution of Washington at Stanford University, elevation 100 feet, Mather, 4,600 feet, and Timberline, 10,000 feet. Those races came from Lapland, Finland, southern Sweden, and a climatic transect across California from sea level to 10,000 feet altitude.

All races are cytologically uniform, having $2n = 26$ chromosomes.

All of the European races became vegetatively apomictic (viviparous) in the transplant experiments, though only half of the individuals were strongly so. This phenomenon is not known to occur in the European habitats of this species, nor in the native California races.

Two bases of comparison were employed. They show (a) individual and racial reactions in uniform environments, and (b) environmental influence when one individual is compared in different environments. The former reveal the inherited characteristics; the latter show the non-inherited modifications. The plasticity of individuals and races is also hereditary.

The following reactions were investigated: vigor, including height of tallest stems, number of flowering stems, and cover-diameter; floriferousness, earliness, fruiting, survival, frost injury, and susceptibility to disease.

Five ecotypes are recognized, as follows: (1) a coastal ecotype of western North America from the maritime climate of northern California; (2) an upper montane Sierran ecotype from the cool montane or subalpine climate; (3) an alpine Sierran ecotype; (4) a cold temperate ecotype from northern Europe; and (5) a subarctic ecotype from Lapland.

All of the five ecotypes belong taxonomically to one subspecies, *D. caespitosa* ssp. *genuina*. These morphologically similar ecotypes are differentiated physiologically with the result that they fit widely different environments. In this species, therefore, physiological differentiation has been independent of morphological differentiation.

Taxonomically, three subspecies are recognized in western North America: *D. caespitosa* ssp. *genuina*, *beringensis*, and *holciformis*, all of which have previously been considered to be independent species. It is pointed out that *D. caespitosa* var. *maritima* Vasey may prove to be a fourth subspecies on this coast, and that *D. bottnica* (Wahl.) Trin. is doubtless but a northern European subspecies of *caespitosa*.

The individuals representing the five ecotypes came from climatically different areas, hence they may be referred to as climatic ecotypes. Their distinctive genetic differences stand out in the comparison of their growth and reproductive behavior in each of the three climatic gardens. For example, the coastal ecotype was most vigorous at Stanford, the Sierran ecotypes at Timberline, and the European ones at Mather.

The theory and practical application of the concept of ecotype should contribute much toward the solution of problems in natural and applied ecology. Various kinds of ecotypes are discussed.

DEPARTMENT OF BOTANY,
OREGON STATE COLLEGE,
CORVALLIS, OREGON
DIVISION OF PLANT BIOLOGY,
CARNEGIE INSTITUTION OF WASHINGTON,
STANFORD UNIVERSITY, CALIFORNIA

LITERATURE CITED

- BONNIER, GASTON. 1890. Cultures expérimentales dans les Alpes et dans les Pyrénées. *Rev. gén. de botanique* 2: 513-546.
- BRAUN-BLANQUET, J. 1932. *Plant sociology: the study of plant communities*. McGraw-Hill. New York.
- CLAUSEN, J., D. D. KECK, AND W. M. HIESEY. 1940. Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. *Carnegie Inst. Washington Pub. No. 520*.
- , ———, AND ———. 1945. Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autopolloidy, with examples from the Madiinae. *Carnegie Inst. Washington Pub. No. 564*.
- , ———, AND E. V. MARTIN. 1942. Experimental taxonomy. *Carnegie Inst. Washington Yearbook No. 41*: 126-134.
- CLEMENTS, F. E. 1929. Experimental methods in adaptation and morphogeny. *Jour. Ecology* 17: 356-379.
- , AND ASSOCIATES. 1937. Adaptation and origin. *Carnegie Inst. Washington Yearbook No. 36*: 222-224.
- , AND ———. 1939. Adaptation and origin. *Carnegie Inst. Washington Yearbook No. 38*: 134-137.
- FLOVIT, KARL. 1938. Cytological studies of arctic grasses. *Hereditas* 24: 265-376.
- GREGOR, J. W. 1930. Experiments on the genetics of wild populations. Part I. *Plantago maritima*. *Jour. Genetics* 22: 15-25.
- . 1931. Experimental delimitation of species. *New Phytologist* 30: 204-217.
- . 1938. Reflections concerning new crop varieties. *Herbage Reviews* 6: 234-239.
- . 1942. The units of experimental taxonomy. *Chronica Botanica* 7: 193-196.
- HAGERUP, O. 1939. Studies on the significance of polyploidy. III. *Deschampsia* and *Aira*. *Hereditas* 25: 185-192.
- HALL, H. M., AND ASSOCIATES. 1926. Experimental taxonomy. *Carnegie Inst. Washington Yearbook No. 25*: 345-346.
- HITCHCOCK, A. S. 1935. *Manual of the grasses of the United States*. U. S. Dept. Agric. Misc. Pub. 200.
- JENKIN, T. J. 1922. Notes on vivipary in *Festuca ovina*. *The Bot. Soc. and Exchange Club of British Isles Report*. Vol. 6: 418-431.
- SINSKAIA, E. N. 1928. The oleiferous plants and root crops of the family Cruciferae. *Bull. Appl. Bot., Genet., and Plant Breed.* 19(3): 1-554. (In Russian.) Summary. (In English) pp. 555-619.
- . 1931. The study of species in their dynamics and interrelation with different types of vegetation. *Bull. Appl. Bot., Genet. and Plant Breed.* 25(2): 50-97.
- STAPLEDON, R. G. 1928. Cocksfoot grass (*Dactylis glomerata* L.): ecotypes in relation to the biotic factor. *Jour. Ecology* 16: 71-104.
- STEBBINS, G. L., JR. 1941. Apomixis in the angiosperms. *Bot. Rev.* 7: 507-542.
- TEDIN, OLOF. 1925. Vererbung, Variation und Systematik in der Gattung *Camelina*. (With summary in English.) *Hereditas* 6: 275-386.
- TURESSON, GÖTE. 1922a. The species and the variety as ecological units. *Hereditas* 3: 100-113.
- . 1922b. The genotypical response of the plant species to the habitat. *Hereditas* 3: 211-350.
- . 1925. The plant species in relation to habitat and climate. Contributions to the knowledge of genecological units. *Hereditas* 6: 147-236.
- . 1927. Contributions to the genecology of glacial relics. *Hereditas* 9: 81-101.

PLANT NUTRITION IN RELATION TO DISEASE DEVELOPMENT.

I. CABBAGE YELLOWS¹

J. C. Walker and W. J. Hooker

THE FIRST critical work on the relation of host nutrition to development of a vascular *Fusarium* disease was carried out by Neal (1927) with cotton wilt (*Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder & Hansen). In the greenhouse he secured a reduction of percentage of diseased plants in the nutrient solution which contained the highest percentage of potassium. In field trials by Miles (1936) in Mississippi over a period of years it was shown that, particularly on potassium-deficient soils, high potash fertilizer consistently reduced the amount of cotton wilt. Confirmatory results in Arkansas were secured by Young and Tharp (1941). In both States it was noted that a relative increase in nitrogen and/or phosphorus on potassium-deficient soil in-

creased wilt. This might have been due to the direct influence of these last two elements upon the disease, or it might have been explained by the fact that the increases really produced a relatively greater deficiency of potassium. In working with cotton wilt, both Miles (1936) and Young and Tharp (1941) pointed out the influence of environmental factors on the nutrition effect. Greater differences between nutrient levels were secured in a relatively susceptible variety, and the differences were smaller and of less significance when a highly resistant variety was used. Stoddard (1942) reported that muskmelon wilt (*F. oxysporum* f. *melonis* (Leach & Currence) Snyder & Hansen) was increased in sand culture when a relatively low potassium and a relatively high nitrogen level were used in the nutrient.

Schroeder and Walker (1942) studied the relation of host nutrition to the development of *Fusarium* wilt (*F. oxysporum* f. *pisi* (Linford) race 1 Snyder & Hansen) of garden pea. A resistant and

¹ Received for publication January 22, 1945.

Investigation supported jointly by the Department of Plant Pathology, University of Wisconsin, with the aid of a grant from the Wisconsin Alumni Research Foundation, and by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

a susceptible variety were grown in inoculated quartz sand maintained at 21°C., which was near the optimum for the host and below the optimum for the pathogen, and at 27°C. which was optimum for the pathogen and above the optimum for the host. At 21° there was no difference in disease development due to nutrient concentration under mid-winter conditions, when days were short and light intensity low. On longer days with higher light intensity there was a decrease in wilt development with increase in nutrient concentration. This was more marked in the susceptible variety. At 27° where cortical decay of the roots as well as vascular invasion and wilt occurred, both varieties showed an increase in disease development, especially cortical necrosis, with increase in nutrient concentration. In the long-day test at 27° the disease declined from the lowest concentration to the next higher concentration but increased markedly at the two still higher concentrations used. It was obvious in the study that the two distinct phases of the disease responded differently to host nutrition. One of these, vascular wilt, was that characteristically observed in nature; the other, cortical root decay, occurred particularly at optimum temperatures for the pathogen and under the conditions of heavy inoculation in quartz sand. Only when an environment conducive to vascular wilt alone was used did increase in nutrient concentration progressively reduce disease development. This study illustrated the complexity which may be encountered in disease development when various controlled environments are used.

The present paper is a report of a study of the influence of host nutrition on the development of another vascular *Fusarium* disease, cabbage yellows (*Fusarium oxysporum* f. *conglutinans* (Wr.) Snyder & Hansen). Cabbage yellows, in common with other vascular *Fusarium* diseases, is influenced markedly by soil temperature. In studies of constant soil temperatures the disease has been shown to increase with temperature to an optimum at about 27°C. (Walker and Smith, 1930). Two types of host resistance have been defined. One of these, herein referred to as Type A, is inherited as a single-factor dominant character (Walker, 1930). It is expressed as complete resistance or immunity, except at constant soil temperatures of 26° to 28°, where atypical symptoms may develop but no invasion of the plant occurs except at the extremities of the root system (Smith and Walker, 1930; Anderson and Walker, 1935). The second, herein referred to as Type B, is controlled by an undetermined number of genes (Anderson, 1933; Blank, 1937). It constitutes successful-commercial control in some varieties but it is not a completely fixed character and increasing percentages of diseased plants occur with increase in soil temperature (Walker and Smith, 1930). It is probably comparable, in inheritance and reaction to temperature, with the type of *Fusarium* resistance which occurs in most resistant varieties of cotton.

METHODS.—For the purposes of this study, strains of the host representing extreme susceptibility, Type A resistance, and Type B resistance were used. For the susceptible host a selected line from an uncommon variety, secured under the name of Smith's Pride, was used. Wisconsin Hollander was used as Type B resistant variety, since it had been shown to carry only this type of resistance (Anderson, 1933). Wisconsin Ballhead was used as the Type A resistant variety, since it had been developed as a homozygous line for this type of resistance (Walker and Blank, 1934).

The studies were conducted in washed quartz sand placed in 8-inch varnished greenhouse pots and watered, by a continuous-drip method, with the desired nutrient. Pots were equipped with a siphon drain as described by Pryor (1940). The nutrient solutions used were the same as described by Smith and Walker (1941). In studies of salt concentration the basal solution, referred to hereafter as 1H (Hoagland's solution), was reduced in concentration of elements to one-twentieth (0.05H), one-tenth (0.1H), and one-half (0.5H) and increased by two times (2H) and by three times (3H). At one or another salt concentration one of the elements was increased (N, 1.15:1; P, 2.0:1; and K, 1.28:1); these solutions are referred to herein as +N, +P, and +K, respectively. Solutions were also used in which either N, or P, or K was omitted; these solutions are referred to herein as —N, —P, and —K, respectively. In solutions with high salt concentrations NaCl was added so that the proportions of the major elements could be varied without changing the total salt concentration.

Seed was sown in quartz sand watered with the basal solution. Seedlings were transplanted two to three weeks later, usually 18 plants per pot. Plants were inoculated one to three weeks after transplanting. Two or more replicate pots were used per treatment and their position was randomized in each replicate block on the greenhouse bench. The yellows organism was grown on Czapek solution. The fungus mats were filtered off, macerated in a Waring Blendor, and suspended in a 0.1 per cent solution of glucose. A 100-cc. aliquot of the fungus suspension was added to each pot to be infested, and equal amounts of 0.1 per cent glucose solution were added to the control pots. Two greenhouses were used, regulated so that the sand was maintained at approximately 19°C. and 25°, respectively. The first of these was about 4° above the minimum for disease development and at this temperature the disease progressed in young seedlings relatively slowly. The second was about 2° below the optimum and the disease developed very rapidly in susceptible plants. Soon after the appearance of yellows in any pot, disease records were taken at intervals of three to five days. Each plant was placed in one of three classes: (1) healthy, (2) diseased (but still alive), (3) killed by yellows. For each pot a disease index was calculated for the population on a scale in which 0 represented all plants healthy and 100 all plants dead.

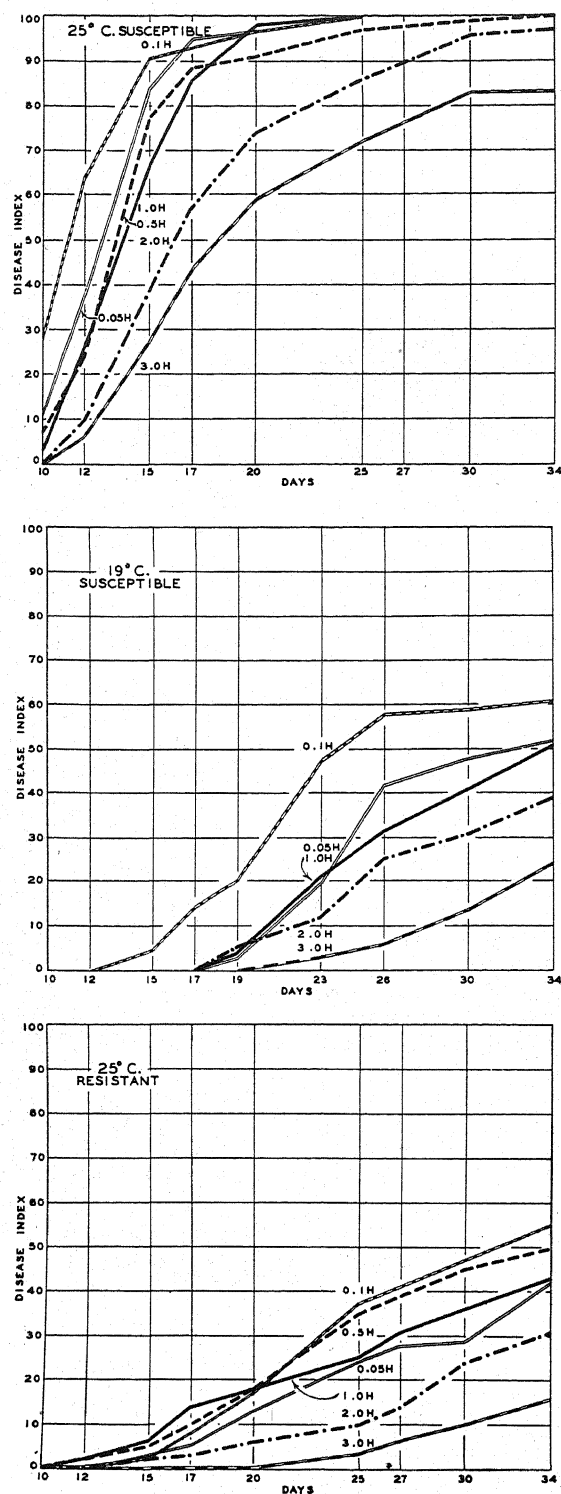


Fig. 1. The influence of salt concentration of the nutrient solution on the development of yellows in a very susceptible host at 19°C. and 25°C. and in a moderately resistant host (Type B) at 25°C. The basal solution is indicated at 1H; 2H and 3H indicate solutions 2 and 3 times, respectively, the salt concentration of the basal solution; 0.5H, 0.1H, and 0.05H are one-half, one-tenth, and one-

EXPERIMENTAL RESULTS.—*Salt concentration.*—Nutrient solutions varying from .05H to 3H were applied to susceptible, Type A resistant, and Type B resistant cabbage plants at 19°C. and at 25°. The results of two experiments with susceptible plants are represented in two of the graphs in figure 1. At the lower temperature the disease appeared first and progressed most rapidly in plants supplied with the 0.1H nutrient. Development with the 0.05H and 1H solution was in each case less rapid. From 1H to 3H, inclusive, there was a marked decrease in disease with increase in concentration. At 25° all disease curves were higher than the highest curve at 19°. This demonstrated very clearly that temperature was somewhat more important than nutrition in its effect upon disease development. Moreover, at 25°, while the disease development curves had the same relation to one another, they were closer together after 20 days than at 19°. This showed that, when the temperature was the more favorable for disease development, the influence of nutrient concentration upon disease development was less evident.

In a second experiment at 25°C. the infested sand of a previous experiment was used. The same trends in the disease curves were secured. The most rapid development was with 0.1H solution; it was slightly less rapid at .05H and progressively slower up to and including 3H. In a second experiment at 19°C., 0.1H, 0.5H, 1H, 2H, and 3H solutions were used. Six replicate pots for each treatment were included. The curves assumed the same general slope as in the 19° experiment shown in figure 1, although in this experiment the disease developed more rapidly at 0.5H than at 0.1H. The disease indices at 33 days after inoculation were treated statistically by the analysis of variance method. The indices at 0.1H and 1H were significantly lower than that at 0.5H, while those at 2H and 3H were significantly lower than that at 1H.

The response of Type B resistant plants may be considered next. As already indicated (Walker and Smith, 1930), this type of host resistance is expressed less effectively as the soil temperature increases. At 19°C. very few of the resistant plants became diseased and, therefore, no measurable effect of nutrient could be secured. At 25° the disease developed more rapidly but much less so than in the susceptible plants at 25°. The disease curves for a Type B resistant series at 25° are shown in figure 1. It was of interest to note that disease development curves for four of the five nutrient concentrations followed closely the shape of the curves for the susceptible host at 19°. In other words, inherent Type B resistance in Wisconsin Hollander had about the same repressive effect on disease development as the lowering of temperature from 25° to 19° had in the susceptible variety. Moreover, when the rate of disease development was retarded either by temperature as in the susceptible host, or by inherent resistance, respectively, the concentration of the basal solution.

ance as in the Type B host, the effect of further reducing disease development by increasing nutrient concentration was greatest. When both temperature (19°) and inherent resistance in the Type B host combined to repress the disease, the effect of nutrition level upon disease development could not be measured.

TABLE 1. *The effect of salt concentration on growth of uninoculated cabbage as indicated by average fresh weight of plant tops.*

Nutrient	Exper. 1 ^a 19°	Exper. 2 19°	Exper. 3 ^a 25°
	gm.	gm.	gm.
0.05H	2.27	...	7.10
0.1H	6.44	4.49	11.10
0.5H	...	10.11	22.82
1H	16.53	10.15	17.56
2H	19.03	8.68	23.23
3H	14.27	7.13	7.82

^a The disease development data from experiment 1 are shown in the center graph of figure 1; those from experiment 3 are shown in the upper graph of figure 1.

In Type A resistant plants, as pointed out earlier in this paper, the yellows disease does not develop at soil temperature below 26°C. It was not expected, therefore, that the disease would be noted in these experiments at 19° or at 25°, unless variation in nutrient concentration had a marked effect upon the expression of this resistance. No disease did appear, showing that Type A resistance is not greatly influenced by nutrient level, if at all, and establishing still more firmly the fact that Type A and Type B resistance to yellows, although phenotypically similar in their expression under certain environmental conditions, are distinct physiologically as well as genotypically.

It was of interest to compare the effect of salt concentration upon the growth of cabbage and to determine whether or not the retardation of disease was brought about by any discernible influence of nutrition on host plant. The uninoculated plants

were cut at the soil level and the fresh weight of tops was recorded in three experiments. These are presented in table 1. It was found that growth increased with salt concentration from 0.05H to 0.5H. The weights of plants grown with 0.5H, 1H, and 2H solutions did not show clear-cut differences and there was in each experiment a decline in weight of tops grown with 3H solution. The pots in experiment 2 were arranged in 4 replications so that the results could be submitted to analysis of variance. It was thus shown that no significant difference existed between the weights at 0.5H, 1H, and 2H, but that the weights in that group were significantly greater than those at 0.1H and 3H. Thus there was a steady decline in disease development from 0.5H to 3H but a significant decline in growth only at 3H. At the lower end of the nutrient range there was still less sign of correlation between disease development and growth of the host. In experiment 3 there was a steady increase in growth from .05H to 0.5H but the most rapid disease development was with the 0.1H nutrient level.

Salt balance.—Nutrient solutions in which either the N, P, or K elements were increased or omitted were studied in relation to disease development. The results of six experiments are brought together in table 2. In the first three experiments the susceptible variety was grown at 19°C. The balance was varied at the 0.1H concentration in one experiment and at the 2H level in the other two. As may be expected from the results already presented, the disease developed more rapidly in solutions at the 0.1H level. The greatest deviation from the basal solution in increase of rate of disease development was in the —K solutions. The disease also developed more rapidly in two out of three cases with the +N solution. In one case the disease developed more rapidly in +P than in basal but in the other two experiments and in all three cases in +K there were no appreciable differences. The disease development rate was consistently slower in the —P solutions than in the basal solutions and the same was true with the —N solutions in two out of three experiments. When the

TABLE 2. *The relation of salt balance in the nutrient solution to development of yellows.*

Exper. number	1	2	3	4	5	6			
Type of host	Susc.	Susc.	Susc.	Susc.	Susc.	Type B Res.			
Temperature	19°	19°	19°	25°	25°	25°			
Nutrient conc.	0.1H	2H	2H	1H	1H	1H			
Duration (days).....	35	36	42	15	32 ^a	15	32 ^a	Average	
Nutrient balance.....	Disease indices							index	
Basal	75	40	53	67	100	62	94	41	56
+N	82	56	46	80	100	76	100	42	64
—N	65	50	42	49	93	67	98	11	47
+P	97	39	48	95	100	82	100	36	66
—P	68	18	41	44	88	64	100	5	40
+K	77	41	58	81	100	69	100	24	58
—K	87	61	83	93	100	71	98	31	71

^a Not included in calculation of the average index.

susceptible variety was grown at 25°C., the same trends were to be seen at 15 days as at 19° after 36

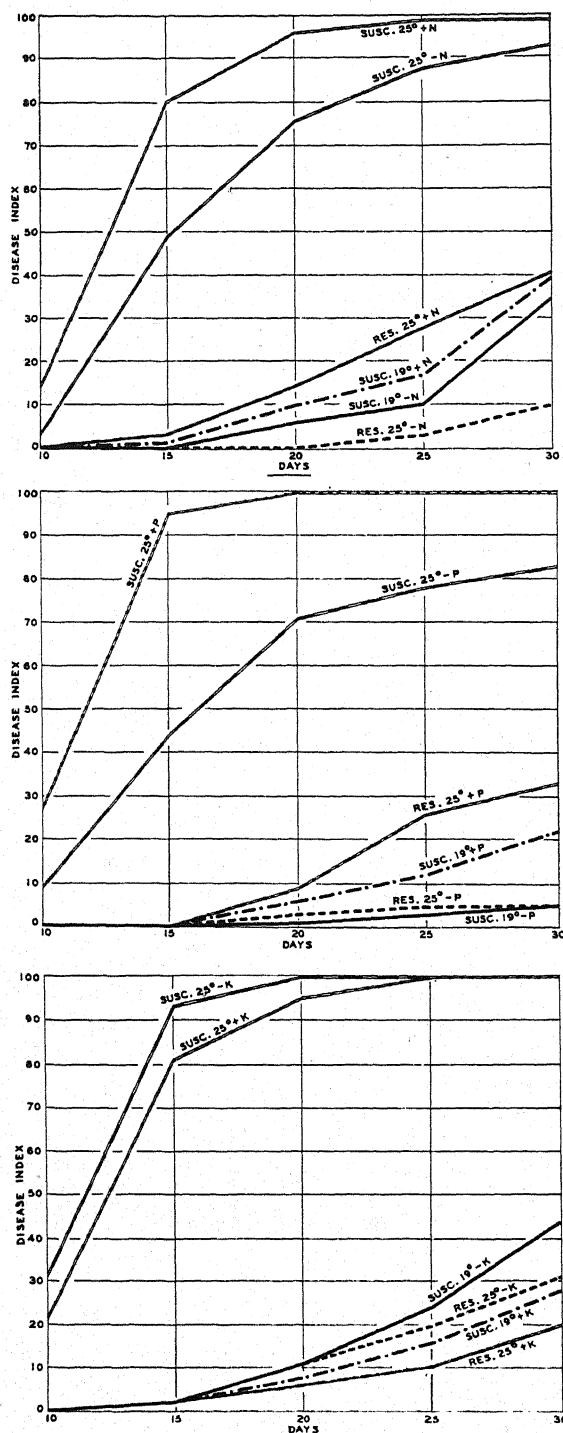


Fig. 2. The relation of salt balance to the development of cabbage yellows. Effects of variations in N, P, and K, respectively, are shown in the three charts. The data for the susceptible variety at 25° are from experiment 4 (table 2); for the susceptible variety at 19°, from experiment 2; and for the Type B resistant variety, from experiment 6.

to 42 days. At 32 days at 25°, however, the rapid progress of the disease erased most of the differences in disease index which had been evident earlier. When the Type B resistant variety was grown in the + and - solutions at 19° the disease development was meager and no differences were evident. At 25°, however, some striking effects were observed (experiment 6). The disease was retarded in -N and -P solutions and in this respect results were similar to those at 19° with the susceptible variety. The disease index in -K was greater than in +K but both were lower than for the basal solution. The daily trends of disease development in the + and - solutions in experiments 2, 4, and 6 are illustrated in figure 2.

It was shown, therefore, that when temperature was optimum for the disease and inherent host resistance was lacking, variously balanced nutrient solutions had only a small influence upon the rate of disease development. When disease development was retarded by lowering the temperature in the susceptible host or by inherent Type B resistance at the optimum temperature, there was usually a decrease in disease development in -N and -P solutions and an increase in -K solutions. When low temperature and Type B resistance combined to retard disease development no measurable effect of nutrient balance was observed. When Type A resistant plants were used, change in neither temperature nor nutrient balance resulted in any development of disease.

Effect of nutrient solutions on growth of the pathogen.—Since the yellows organism was added directly to the quartz sand irrigated with various nutrient solutions, it was important to know what influence the solutions might have upon the increase of inoculum before infection. Each nutrient solution used was made up with 2 per cent agar, each with 3 levels of dextrose (0.5, 1.0, and 2.0 per cent). Petri dish cultures of each were made in triplicate and radial expansion of colonies measured at the end of four days. No significant differences were found between amounts of growth on any of the agars. This indicated that differences in disease development in plants grown in various solutions were due to the effect upon the host rather than to a direct effect of the nutrient solutions upon the organism before establishment of its parasitic relation to the host.

Discussion.—Development of a parasitic disease may be considered in three phases, during each of which environmental factors may become influential. In the first phase, which may be termed the predispositional phase, conditioning of host and parasite are involved. The second phase, which may be referred to as the incitant or inception phase, is concerned with penetration by and parasitic establishment of the pathogenic organism. The third phase, which may be termed the developmental phase, constitutes the period of interaction of host and parasite and the resultant development of disease symptoms and effects in the former. This study

is concerned primarily with the developmental phase of cabbage yellows. Three influential factors—temperature, inherent host resistance, and nutrition—were studied. It is obvious that in nature these factors, with others, act collectively rather than independently. The influence of any one, therefore, may be conditioned somewhat by the others.

In this study the interaction of host resistance was removed by the use of a very susceptible strain of cabbage. A rise in temperature from 19°C. to 25° greatly accelerated disease development. An increase in salt concentration of the nutrient (except at the lowest levels) resulted in a retardation of disease development in the susceptible variety but the interaction of nutrition was most effective at the lower temperature which was least influential in promoting disease development. Likewise the effects of —N, —P, and —K solutions were greater in this variety when the effect of temperature in promoting disease was least.

The influence of inherent host resistance of an intermediate degree was studied by the use of Type B resistant Wisconsin Hollander cabbage. With the introduction of this third factor the interaction of temperature and nutrition was modified. Where temperature and inherent host resistance both suppressed the disease, nutrition was effective neither in increasing nor in decreasing disease development. At 25°C., where temperature hastened disease development, the suppression of disease by inherent resistance was less effective than at 19°. In this situation (25°) nutrition again became effective in enhancing or suppressing disease development according to its level or balance.

When Type A resistant Wisconsin Ballhead cabbage was used the influence of the high degree of inherent host resistance excluded any effect of either temperature or nutrition upon disease development.

It may be concluded, then, that under the conditions of these experiments with young plants in the greenhouse, nutrition did have an effect upon disease development in that increase in salt concentration, except at the low levels, progressively retarded disease development while low nitrogen and low phosphorus tended to retard, and low potassium tended to enhance, disease development. The extent of nutritional effect, however, was conditioned by the interaction of temperature and the degree of inherent host resistance. It appears, therefore, that cabbage yellows has the same type of response to nutrition as has been recorded for other vascular *Fusarium* diseases. The reverse in nutritional effect at high temperature noted by Schroeder and Walker (1942) with *Fusarium* wilt of pea was not observed with yellows. Their result was associated with a modification of the pathogenic habit of the organism from that of a primarily vascular invader to one causing cortical root decay as well. The fact that no such change in host-parasite relation occurred at 25°C. with cabbage yellows probably accounts for the difference in results with the two diseases.

Suppression or enhancement of yellows development by change in salt concentration or balance was not found to be correlated with rate of growth of the host.

SUMMARY

The rate and severity of development of the yellows disease (*Fusarium oxysporum* f. *conglutinans* (Wr.) Snyder & Hansen) were studied in young cabbage plants growing in quartz sand cultures irrigated with nutrient solutions varying in salt concentration and in balance of nitrogen, phosphorus and potassium. Plants of a very susceptible strain, an intermediate resistant strain, and a highly resistant strain of cabbage were used. The salt concentration of the basal Hoagland solution was decreased by one-half (0.5H), one-tenth (0.1H), and one-twentieth (0.05H) and increased by two times (2H) and by three times (3H).

There was usually an increase in growth up to 1H or 2H and then a decline at 3H. In one experiment, set up so that the data could be analyzed statistically, there was not a significant difference between the fresh weight of tops of uninoculated plants at 0.5H, 1H, and 2H, but the weights in that group were significantly higher than those at 0.1H and 3H. Except at the low concentrations there was a progressive decline in rate of disease development in the susceptible strain with increase in salt concentration. The rate of disease development was slower at 19°C. than at 25°. While the disease development curves had the same relation to each other at 25° as at 19°, they were closer together after 20 days at the former temperature. With intermediate resistant plants inherent host resistance suppressed the disease almost completely at 19° and no effect of salt concentration could be measured; at 25° the curves for four out of five nutrient concentrations followed closely the shape of those for the susceptible strain at 19°.

When potassium was omitted from the solution the rate of disease development increased in the susceptible strain at 19°C. and 25°. When nitrogen and when phosphorus were omitted the rate of disease development decreased in the susceptible strain at 19° and 25° and in the intermediate resistant strain at 25°.

In the highly resistant strain no signs of disease developed at any salt concentration or at any balance of nitrogen, phosphorus, or potassium in the nutrient.

Suppression or enhancement of yellows development by change in salt concentration or balance was not found to be correlated with rate of growth of the host.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN, AND
DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL
ENGINEERING,
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
MADISON, WISCONSIN

LITERATURE CITED

- ANDERSON, M. E. 1933. *Fusarium* resistance in Wisconsin Hollander cabbage. Jour. Agric. Res. 47: 639-661.
- , AND J. C. WALKER. 1935. Histological studies of Wisconsin Hollander and Wisconsin Ballhead cabbage in relation to resistance to yellows. Jour. Agric. Res. 50: 823-836.
- BLANK, L. M. 1937. *Fusarium* resistance in Wisconsin All Seasons cabbage. Jour. Agric. Res. 55: 497-510.
- MILES, L. E. 1936. Effect of potash fertilizers on cotton wilt. Mississippi Agric. Exper. Sta. Tech. Bull. 23, 21 p.
- NEAL, D. C. 1927. Cotton wilt: a pathological and physiological investigation. Annals Missouri Bot. Gard. 14: 359-407.
- PRYOR, D. E. 1940. The effect of some mineral nutrients on the development of clubroot of crucifers. Jour. Agric. Res. 61: 149-160.
- SCHROEDER, W. T., AND J. C. WALKER. 1942. Influence of controlled environment and nutrition on the resistance of garden pea to *Fusarium* wilt. Jour. Agric. Res. 65: 221-248.
- SMITH, P. G., AND J. C. WALKER. 1941. Certain environmental and nutritional factors affecting *Aphanomyces* root rot of garden pea. Jour. Agric. Res. 63: 1-20.
- SMITH, ROSE, AND J. C. WALKER. 1930. A cytological study of cabbage plants in strains susceptible or resistant to yellows. Jour. Agric. Res. 41: 17-35.
- STODDARD, D. L. 1942. *Fusarium* wilt of cantaloupe and studies on the relation of potassium and nitrogen supply to susceptibility. Trans. Penin. Hort. Soc. 31: 91-93.
- WALKER, J. C. 1930. Inheritance of *Fusarium* resistance in cabbage. Jour. Agric. Res. 40: 721-745.
- , AND L. M. BLANK. 1934. *Fusarium*-resistant Danish Ballhead cabbage. Jour. Agric. Res. 49: 983-989.
- , AND ROSE SMITH. 1930. Effect of environmental factors upon the resistance of cabbage to yellows. Jour. Agric. Res. 41: 1-15.
- YOUNG, V. H., AND W. H. THARP. 1941. Relation of fertilizer balance to potash hunger and the *Fusarium* wilt of cotton. Arkansas Agric. Exper. Sta. Bull. 440, 24 p.

FACTOR Z_2 AND GAMETIC REPRODUCTION BY PHYCOMYCES¹

William J. Robbins and Mary Bartley Schmitt

IN EARLIER papers from this laboratory the favorable action, in the presence of thiamine, of an extract of white potatoes and other natural substances on the germination, mycelial growth, and gametic reproduction of *Phycomyces blakesleeanus* has been reported (Robbins, 1939, 1940, 1941). Robbins and Hamner (1940) demonstrated that at least two factors were involved. One (factor Z_1) was adsorbed on charcoal; the other (factor Z_2) was present in the filtrate from charcoal-treated extracts. Factor Z_1 was identified as hypoxanthine (Robbins and Kavanagh, 1942b; Robbins, Kavanagh and Kavanagh, 1942; Robbins, 1943). As the studies were continued, it appeared that the relation of these factors to spore germination, to mycelial growth, and to gametic reproduction should be considered separately. The present paper reports our conclusions on factor Z_2 so far as gametic reproduction is concerned.

Our earlier results showed that *Phycomyces* produced few or no progametes at 26°C. when the two strains were inoculated at opposite edges of a 10 cm. Petri dish containing a basal medium composed of mineral salts, dextrose, asparagine, thiamine and agar. At 20°C. under the same conditions zygotes were produced. The addition of potato extract to the basal medium increased the number of zygotes at 20°C. and induced the formation at 26°C. of yellow progametes. These, however, did not mature at the higher temperature. The filtrate from charcoal-treated potato extract acted on gametic reproduction in much the same way as the original extract. If the inoculations of the two strains were

separated by 1 or 2 cm. instead of by the diameter of the Petri dish, progametes developed at 26°C. on the basal medium.

We have been concerned in determining why the temperature of incubation, the addition of potato extract (and its filtrate fraction), and the distance between inocula influence gametic reproduction of *Phycomyces* on our basal medium.

It is possible to explain each of these phenomena by assuming the existence of one or more factors essential for gametic reproduction. On this basis the effect of the higher temperatures would be caused by a failure of the organism to make the essential factors in as great a net quantity at 26°C. as at 20°C. Potato extract might contain the essential factors which are not present in the basal medium. If present in small quantity in the spores used as inoculum, the factors might be used up or dissipated before the mycelia meet if they grow for some distance.

Our object in this investigation was to determine whether any specific compounds were concerned in the phenomenon described, or whether the results were due to some other cause; that is, to identify factor Z_2 for gametic reproduction.

METHODS.—The procedure followed, unless otherwise noted, was as follows:

Phycomyces blakesleeanus was grown at 26°C. in 2 × 10 cm. Petri dishes of pyrex glass. Each dish contained 20 ml. of a basal medium² or the

² This medium contained per liter 1.5 g. KH_2PO_4 , 0.5 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2 g. asparagine, 50 g. dextrose, 500 mμ moles thiamine, 500 mμ moles hypoxanthine, and 10 g. purified agar (Robbins and Ma, 1943). Supplementary mineral elements were added as follows in p.p.m.: 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn.

¹ Received for publication February 12, 1945.

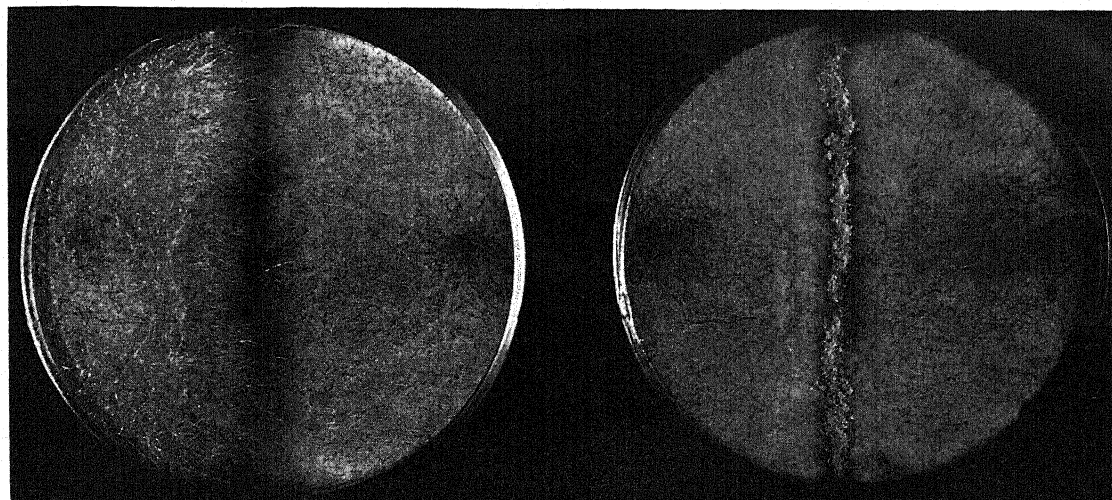


Fig. 1. Effect of glutamic acid on gametic reproduction of *Phycomyces* at 26°C. Left, basal medium; right, basal medium plus 10 mg. d-glutamic acid, neutralized with CaCO_3 . The mycelia have been pulled aside in both plates to show line of union. Note line of progametes in plate with glutamic acid. Age 6 days.

same medium supplemented by various additions which are given in the text in connection with specific experiments. Each dish was inoculated at one end of a diameter with a drop of a spore suspension of the (+) strain, and at the other end with the (—) strain. The original inocula were separated by a distance of about 9 cm. To insure maximum germination the spore suspensions were heated for 30 min. at 50°C. Observations were made at intervals on growth, on the distance separating the developing mycelia, and on the completeness with which the two mycelia had joined; it is obvious that progametes and zygotes would be produced only if the two mycelia met. Final observations on progamete production were made at the end of 5 or 6 days. The progametes appeared as a yellow line of various lengths and widths, depending upon the medium, and with few exceptions reached their maximum development within 5 or 6 days. At 26° the progametes did not form mature zygotes.

All glassware was cleaned by treatment with a chromic sulfuric acid mixture, followed by thorough rinsing with tap water and distilled water. The chemicals were of the customary C.P. grade. The asparagine was treated with charcoal and recrystallized from alcohol.

RESULTS.—*Protein hydrolysates.*—Among the substances tested for factor Z_2 activity were hydrolysates of gelatine, egg albumen, and casein. All of these exerted a favorable effect though none was as active on a dry weight basis as potato extract.³ Since the major constituents of these hydrolysates were amino acids, we prepared and tested mixtures of amino acids which approximated the composition of the hydrolysates of the natural products. These "synthetic" protein hydrolysates also were of benefit, though less so than potato extract.³ These re-

³ In some instances the hydrolysates were superior to potato extract when smaller quantities (1 or 2 mg. per plate) were compared.

sults indicated that the effect of the individual amino acids included in our synthetic hydrolysates should be determined.

Amino acids.—Ten amino acids which are common to egg albumen, casein and gelatine were selected and divided into two groups, A and B.⁴ Each group was treated with excess CaCO_3 , filtered sterile and added to the basal medium in amounts of 20 mg., 10 mg., 5 mg., and 1 mg. per dish. Mixture A was inactive, mixture B was active and, in the smaller amounts, superior to equal weights of potato extract.

These results suggested that the material present in the protein hydrolysates and favorable for gametic reproduction under our conditions was one or more of the four amino acids included in mixture B.

We, therefore, prepared mixtures containing three of the four amino acids, omitting one of the four in turn. These were used in the proportions in mixture B and in amounts equivalent to 20 mg. or 10 mg. of the mixture per plate. Beneficial effects were observed whenever glutamic acid⁵ was included and no progametes were obtained with a combination of leucine, isoleucine and proline.

Glutamic acid.—Glutamic acid added by itself to the basal medium exerted a beneficial effect; in fact, up to 20 mg. per plate (larger amounts were not tested), it was more effective than equal weights of potato extract (fig. 1). On the other hand, leucine, isoleucine, proline, valine, tryosine and glycine,

⁴ Mixture A contained equal weights of d-arginine HCl, d-lysine HCl, dl-phenylalanine, dl-threonine, dl-valine and dl-serine. Mixture B contained four amino acids in the following proportions by weight: 2.5 l-leucine, 7.5 d-isoleucine, 5.0 l-proline and 15 d-glutamic acid.

⁵ The glutamic acid was shaken with CaCO_3 and the excess removed by filtration. This procedure was followed whenever glutamic acid was used. The addition of unneutralized glutamic acid was ineffective. Glutamic acid neutralized with NaOH was as active as that neutralized with CaCO_3 .

added individually to the basal medium at the rate of 10, 5 or 2 mg. per plate, had little or no effect.

Different amounts of asparagine.—The beneficial effect of glutamic acid was not caused by its nitrogen content. This is suggested by the inactivity of other amino acids which would also increase the nitrogen in the medium. Some of these were capable of serving as sources of nitrogen to *Phycomyces* (Schopfer, 1937). We supplemented the basal medium also with 0.5, 1.0, 5.0 and 10 mg. of asparagine per plate without influencing the gametic reproduction.

Effect of other substances.—Although glutamic acid alone of the ten amino acids investigated favorably influenced gametic reproduction, it appeared desirable to test other substances, particularly other organic acids. Each substance was used at 10 mg., 5 mg. and 2.5 mg. per plate; and the activity was compared with that of equal weights of glutamic acid and of potato extract. Pyruvic acid, glutaric acid, and aspartic acid were treated with excess CaCO_3 filtered, and autoclaved before being added to the sterile basal medium. Glutamine⁶ was treated with excess CaCO_3 and filtered sterile. All the other compounds were neutralized with NaOH and filtered sterile before being added to the sterile basal medium. Glutamine was ineffective. Pyruvic, glutaric, aspartic, fumaric, malic, succinic, tartaric, citric, lactic, propionic, pimelic, hippuric, azelaic, malonic, hydrocinnamic, valeric, butyric, isobutyric, and isocaproic acids were all effective. Some of them were as active as glutamic acid in amounts of 10 mg. per plate, and more effective than glutamic acid at 5 or 2.5 mg. per plate; others were less effective. Many inhibited to a greater or lesser extent the development of the yellow pigment in the progametes; others (hydrocinnamic and pyruvic acids) intensified the yellow color. Hydrocinnamic, isobutyric, and isocaproic acids at 10 mg. per plate were sufficiently injurious to prevent the (+) and (—)

⁶ This glutamine was generously provided by Dr. Paul György.

strains from growing together; but 5 and 2.5 mg. induced the formation of progametes.

Buffered media.—These results showed that the action of glutamic acid was not specific and suggested that the beneficial effects of the organic acids (including glutamic acid) might be primarily due to their buffer action. We, therefore, modified the basal medium by substituting for the KH_2PO_4 , $\frac{M}{30}$ or $\frac{M}{90}$ mixtures of the monobasic and dibasic potassium phosphates adjusted to give various hydron concentrations. The initial hydron concentrations of these media were pH 4.5 (the basal medium), pH 6.0, pH 6.5, pH 7.0 and pH 8.0. With both amounts of the phosphates, at initial pH 7.0 and pH 8.0 growth was materially reduced; and the two strains did not meet. On the plates at initial pH 6.0 and pH 6.5 lines of progametes were obtained though they were paler and in smaller quantity than on the basal medium supplemented with potato extract.

We also added to the basal medium $\frac{M}{20}$ or $\frac{M}{100}$ phthalate buffers to give initial hydron concentrations of pH 4.6, 5.0 and 6.0. The $\frac{M}{20}$ concentration was injurious at pH 4.6 and 5.0 but at pH 6.0 the progamete production was superior to the check though paler and sparser than with potato extract. With the $\frac{M}{100}$ concentrations, progametes were obtained at all three hydron concentrations.

Various amounts of CaCO_3 (10, 100, 250 and 500 mg. per plate) were added to the basal medium. The gametic reproduction was superior to the check though not as satisfactory as with potato extract.

In none of the buffered media or in those neutralized with CaCO_3 were the progametes as numerous as we obtained with glutamic acid or with potato extract; neither were they as yellow. Nevertheless, the results supported the idea that the failure of *Phycomyces* to produce gametes on our basal medium was associated with the acidity of the medium.

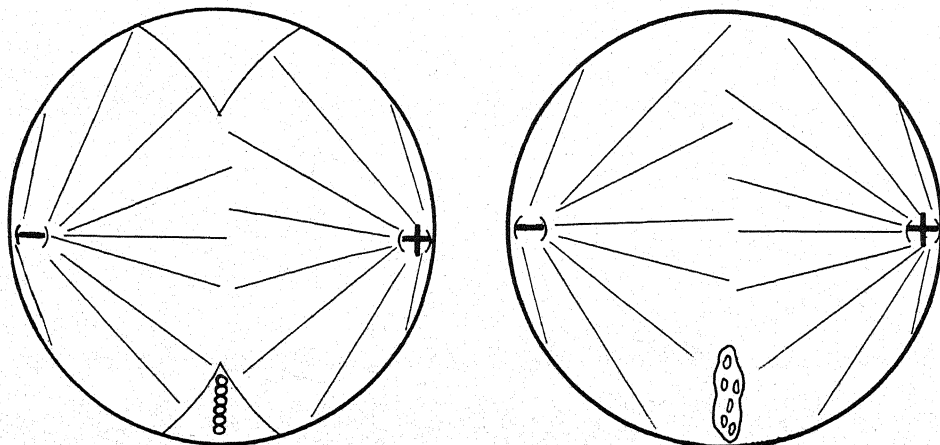


Fig. 2. Diagrams to illustrate application of drops of neutralizing solutions. Left, condition at time drops were applied; right, condition when progametes had developed. See text for details.

TABLE 1. Relation between hydron concentration of medium and development of progametes at 20°C. and 26°C. See text for details.

	3 days	4 days	5 days	6 days
20°C.				
Distance apart (—) or line of union (+) of two mycelia....	—2.0 cm.	+4.8 cm.	+8.7 cm.	...
Line of progametes.....	0	3.3 cm.	6.9 x 0.6 cm.	...
p _H of medium.....	4.5	4.15	3.5	3.0
Dry weight of mat.....	501 mg.	534 mg.
26°C.				
Distance apart (—) or line of union (+) of two mycelia....	—0.7 cm.	+5.5 cm.	+8.7 cm.	...
Line of progametes.....	0	0	0	...
pH of medium.....	4.35	3.75	3.15	3.1
Dry weight of mat.....	533 mg.	513 mg.

Neutralization of acidity near growing colonies.

—The results with buffered media suggested that it might be possible to produce progametes by neutralizing the acid locally at the edges of the growing mycelia.

The two strains were allowed to grow on the basal medium until they met and formed a line of union extending over about half the diameter of the plate. At one end of the line of union in that portion of the agar not yet covered with mycelium we placed (fig. 2) several drops of water containing excess CaCO_3 , or of solutions of $\frac{M}{15} \text{KH}_2\text{PO}_4$, $\frac{M}{30} \text{Na}_2\text{HPO}_4$, 0.2 N NaOH or a mixture of KH_2PO_4 and Na_2HPO_4 of pH 7.0. Progametes were produced in the area treated with the neutralizing substances and not elsewhere in the plates (fig. 2). The best results were obtained with $\frac{M}{15} \text{K}_2\text{HPO}_4$ and with CaCO_3 .

Measurement of hydron concentrations.—The foregoing results suggested the wisdom of measuring the acidity of the medium on which the two strains were growing and correlating the results with the development of progametes.

In the first experiment (table 1) 60 ml. of the basal medium were used in a Petri dish. Several dishes were inoculated with the (+) and (—) *Phycomyces*; some were incubated at 20°C., others at 26°C. After 3, 4, 5 and 6 days a portion of the agar was removed at the edge of the growing colonies or under the line of union where the mycelia had met, and the pH was determined by means of a glass electrode. At the same time notes were made on the distance separating the (+) and (—) strains or the length of the line of union if they had already grown together. Observations were made also on the production of progametes. On the 5th and 6th days a mycelial mat was stripped from the agar, washed in warm water, dried and weighed. The growth was more rapid at 26° than at 20°. After 3 days the two mycelia at 20° were, on the average, 2.0 cm. apart; at 26° they were 0.7 cm. apart. The pH of the agar was 4.5 at 20° and 4.35 at 26°. By

the 4th day the two mycelia had met at 20°, and formed a line of union averaging 4.8 cm. in length. Progametes had begun to develop extending for 3.3 cm. or about $\frac{3}{4}$ of the length of the line of union. At 26°, although the line of union was 5.5 cm. in length, no progametes had formed. However, the pH of the medium on the 4th day was 4.15 at 20°C. and 3.75 at 26°C. On the 5th day the length of the line of union and of progametes had increased at 20° extending nearly across the plate. The pH had dropped to 3.5. At 26° although the line of union of the two mycelia extended nearly across the plate, no progametes appeared. The pH was 3.15.

These results suggest that a hydron concentration lower than approximately pH 4.0 inhibits progamete production. At the lower temperature (20°) the shift past the critical hydron concentration occurs after the progametes have formed, and at the higher temperature it occurs before their development. At both temperatures the final pH after 6 days was in the vicinity of 3.0, and the vegetative growth as judged by the dry weights was about the same.

In the second experiment 20 ml. of medium were used per dish and we prepared dishes of the basal medium, and of the basal medium plus 20 mg. of potato extract per plate. Some plates of each medium were incubated at 20°C. and at 26°C. In this experiment also the hydron concentration of the basal medium at the time progamete formation began on the 4th day was above 4.0 (pH 4.4) at 20°C. and below 4.0 (pH 3.6) at 26°. The addition of potato extract decreased the acidity of the basal medium. It also increased the rate of growth so that the mycelia at both 20° and 26° in the media supplemented with potato extract had grown together by the 3rd day and had begun to produce progametes (table 2); at this time the pH of the media at 20°C. was 4.9 and at 26°C., 4.6.

It appears therefore that the failure of *Phycomyces* to produce progametes at 26°C. on our basal medium occurs because, by the time the mycelia have met, the acidity of the medium has become too great to permit their formation.

TABLE 2. Relation between hydron concentration of medium and development of progametes at 20°C. and 26°C. on basal medium and basal medium plus potato extract. See text for details.

	2 days	3 days	4 days	5 days	6 days
Basal medium 20°C.					
Distance apart (—) or line of union (+) of two mycelia	—2.2 cm.	+4.8 cm.	+9.0 cm.	+9.0 cm.
Line of progametes.....	0	0	2.6 cm.	7.4 x 0.4 cm.	8.5 x 0.4 cm.
pH of medium.....	4.65	4.65	4.4	3.25	2.9
Dry weight of mat.....	122 mg.	182 mg.	175 mg.
Basal medium 26°C.					
Distance apart (—) or line of union (+) of two mycelia	—1.3 cm.	+3.5 cm.	+8.0 cm.	+8.0 cm.
Line of progametes.....	0	0	0	0	0
pH of medium.....	4.6	4.4	3.6	3.0	3.1
Dry weight of mat.....	181 mg.	183 mg.	209 mg.
Basal medium plus potato extract 20°C.					
Distance apart (—) or line of union (+) of two mycelia	+2.1 cm.	+9.0 cm.	+9.0 cm.	+9.0 cm.
Line of progametes.....	0	few	8.4 x 0.5 cm.	9.0 x 0.6 cm.	9.0 x 0.6 cm.
pH of medium.....	4.9	4.9	4.2	3.3	3.1
Dry weight of mat.....	201 mg.	216 mg.	197 mg.
Basal medium plus potato extract 26°C.					
Distance apart (—) or line of union (+) of two mycelia	+5.6 cm.	+9.0 cm.	+9.0 cm.	+9.0 cm.
Line of progametes.....	0	few	6.8 x 0.2 cm.	9 x 0.2 cm.	9.0 x 0.2 cm.
pH of medium.....	4.85	4.6	3.6	3.3	3.3
Dry weight of mat.....	264 mg.	231 mg.	227 mg.

Buffer action of glutamic acid.—The favorable effect of glutamic acid on the production of pro-

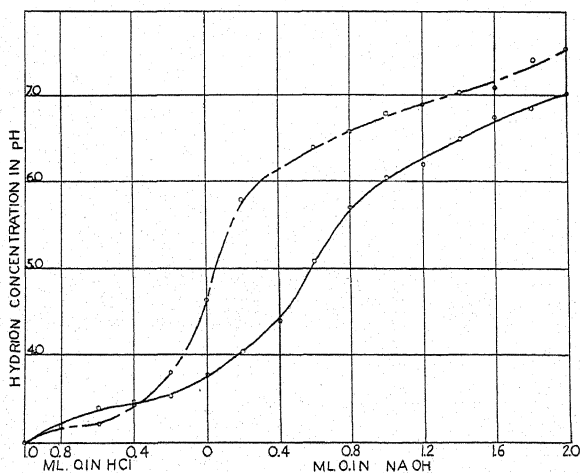


Fig. 3. Titration curve (dotted) of 20 ml. of basal solution (mineral salts, sugar, asparagine), and titration curve of same medium plus 10 mg. of d-glutamic acid, neutralized with CaCO_3 .

gametes under our conditions probably results from its buffer action. Titration curves of our basal nutrient solution, and the same solution plus 10 mg. of glutamic acid per 20 ml., show that the latter is

well buffered in the vicinity of the critical pH 4.0 (fig. 3). The basal solution owes its buffer action chiefly to the phosphate it contains, which is least effective between pH 4.0 and pH 5.0.

Plates with compartments.—We demonstrated the relation between acidity and the production of progametes by another method, one which did not involve the addition of any chemical substances to the basal medium. Three Petri dishes were prepared with partitions of quartz glass 4 mm. high and 2 or 3 mm. wide, which separated the dish into 3 compartments. The partitions were cemented to the bottoms of the dishes. The distance between the partitions in the three plates was 26 mm., 19 mm., and 11 mm. respectively. Sufficient basal medium was poured into each dish to reach the top of the partitions, and the plates were inoculated at opposite edges with the (+) and (—) strains (fig. 4). The temperature of incubation was 26°C.

We assumed that the partitions would prevent acid developed on the side portions of the plates occupied by the growing mycelia from diffusing into the middle portion. When the mycelia grew over the partitions, they would find media unchanged in acidity; and the time before they met would be too short to permit the acidity of the middle portion of the medium to pass beyond the critical point.

In the plate with partitions 26 mm. apart the two strains grew across the partitions and met in

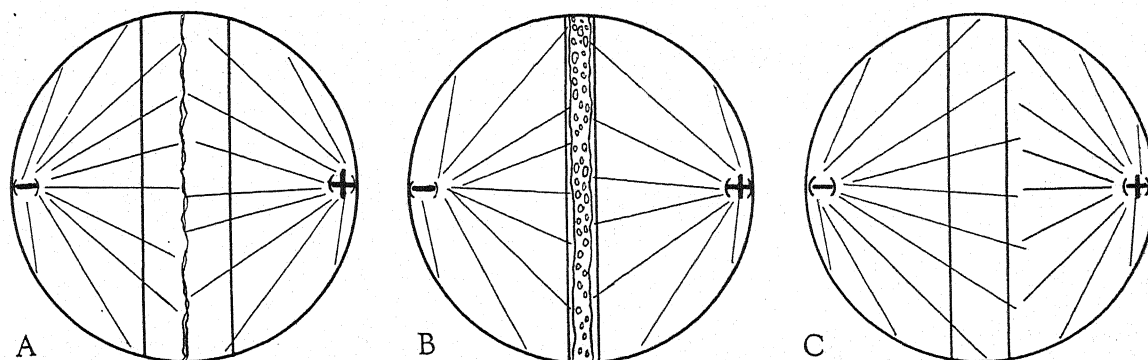


Fig. 4. Results obtained with partitioned plates. Note in A and B line of progametes where mycelia met in medium between partitions; and no progametes in C, where the (—) strain overgrew the middle partition of agar before meeting the (+) strain.

the agar between, producing a line of progametes entirely across the plate and 2 or 3 mm. wide. The same thing occurred in the dish with the partitions 11 mm. apart, but the width of the line of progametes ranged up to 8 or 9 mm. In the third plate the (—) strain grew across the middle portion of the medium and met the (+) strain beyond the second partition; no progametes developed.

DISCUSSION.—The failure of *Phycomyces* to produce progametes on our basal medium at 26°C. when the inoculations were made at opposite edges of a 10 cm. Petri dish occurred because the acidity became great enough to inhibit their production before the (+) and (—) strains met. This critical hydrion concentration was not accurately defined, but appeared to be in the vicinity of pH 4.0. It is important to note that the time factor played an important role in this effect. Even though the pH of the medium ultimately reached 3.0, progametes were formed, provided the acidity at the time the (+) and (—) strains grew together had not fallen below pH 4.0 ±. Any conditions, therefore, which permitted the two strains to meet before the critical hydrion concentration had developed, exerted a favorable effect on gametic reproduction.

The beneficial effect of potato extract was not the result of any specific chemical substance in the nature of a "reproductive" factor. It was effective because it stimulated the growth of both the (+) and (—) strains and exerted some neutralizing action, thus bringing the two strains together before metabolic activity had formed an inhibitory amount of acid. Other substances, for example, neutralized glutamic acid, owed their favorable effects chiefly to their buffering action in the vicinity of the critical pH value and little to an acceleration of growth.

Temperature affected gametic reproduction because there was a slower formation of acid at the lower temperatures (20°) than at the higher (26°). Distance between inocula was a factor to be considered because when the (+) and (—) inocula were close together the mycelia joined before much acid had been formed; when they were farther apart (9 cm.), the time necessary for them to grow to-

gether also permitted the development of an inhibitory amount of acid.

As a corollary to these general statements, we may add that our observations indicate that it requires a greater hydrion concentration to inhibit vegetative growth of this fungus than to prevent the formation of gametes.

At various periods in our investigation, it was possible to present considerable evidence for definite chemical substances functioning as reproductive factors; in other words, to identify factor Z₂ for gametic reproduction by *Phycomyces*. However, we have been forced to conclude that factor Z₂ for progamete formation is not a specific chemical compound but is any factor which keeps the hydrion concentration of the medium from becoming too acid until after gametic reproduction has had an opportunity to occur.

SUMMARY

The failure of *Phycomyces blakesleeana* to form progametes at 26° on a basal medium of mineral salts, asparagine, dextrose and thiamine was found to be caused by the development of too much acidity before the (+) and (—) strains grew together. The addition to the basal medium of a potato extract, of neutralized protein hydrolysates, neutralized glutamic acid and other organic acids favored gametic reproduction because these supplements buffered the medium or increased the rate of growth so that the mycelia met before the critical hydrion concentration was reached. The production of acid was slower at 20°C. than at 26°C., which accounted for the favorable effect of the lower temperature on gametic reproduction. Factor Z₂ for gametic reproduction by *Phycomyces* is not a specific substance, but is any condition which allows the mycelia to join before the critical hydrion concentration has developed in the medium.

NEW YORK BOTANICAL GARDEN
AND
DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY

LITERATURE CITED

- ROBBINS, WILLIAM J. 1939. Growth substances and gametic reproduction by *Phycomyces*. Bot. Gaz. 101: 428-449.
- . 1940. Effect of extracts of *Phycomyces* upon its development. Amer. Jour. Bot. 27: 559-564.
- . 1941. Further observations on Factor Z. Bot. Gaz. 102: 520-535.
- . 1943. Further observations on the specificity of hypoxanthine for *Phycomyces*. Proc. Nat. Acad. Sci. U.S.A. 29: 201, 202.
- , AND K. C. HAMNER. 1940. Effect of potato extracts on growth of *Phycomyces*. Bot. Gaz. 101: 912-927.
- , AND F. KAVANAGH. 1924a. Guanine and factor Z₁, growth substances for *Phycomyces*. Proc. Nat. Acad. Sci. U.S.A. 28: 4-8.
- , AND ———. 1942b. Hypoxanthine, a growth substance for *Phycomyces*. Proc. Nat. Acad. Sci. U.S.A. 28: 65-69.
- , V. W. KAVANAGH, AND F. KAVANAGH. 1942. Growth substances and dormancy of spores of *Phycomyces*. Bot. Gaz. 104: 224-242.
- , AND R. MA. 1943. The relation of certain fungi to thiamine. Bull. Torrey Bot. Club 70: 190-197.
- SCHOPFER, W. H. 1937. Recherches sur le métabolisme de l'azote d'un microorganisme acellulaire (*Phycomyces blakesleeanus* Bgf.). Le rôle des facteurs de croissance. Protopl. 28: 381-434.

ENDOMITOTIC TAPETAL CELL DIVISIONS IN SPINACIA¹

E. R. Witkus

IN MANY angiosperms the tapetal cells of young anthers are uninucleate diploid cells. In mature anthers these cells are frequently binucleate or multinucleate. Diverse and at times conflicting explanations of the origin of this binucleate or multinucleate condition are found in the literature. Earlier authors commonly stated that amitosis is the typical method of nuclear division in tapetal cells but more recent workers, from a study of the developmental stages involved, are in general agreement that these divisions are mitotic rather than amitotic.

Atypical division figures suggestive of amitosis are common in tapetal cells. In 1882 Strasburger found mitotic divisions in the tapetum and stated that the divisions he had earlier described as amitosis were in reality the closely appressed nuclei of binucleate cells. In 1906 Tischler stated that two divisions occurred in the tapetum of certain *Ribes* hybrids. The first was a normal mitotic division but no cell plate was formed and a binucleate cell resulted. These nuclei then simultaneously underwent a second division which was amitotic. In 1933 Smith found that the tapetal cells of *Galtonia candicans* undergo typical mitosis and form binucleate cells with two diploid nuclei. These two nuclei then simultaneously undergo a second division in which certain irregularities occur. During late prophase as the nuclear membrane disappears, both diploid sets of chromosomes congress upon one equatorial plate. As a result of this mitotic division two tetraploid nuclei are formed in the same cell. If the two dividing nuclei do not coalesce in this way, they proceed to metaphase separately and four diploid nuclei are formed, again with no cell plate formation. In some cases because of the close proximity of the two centrally located telophase groups, they may fuse, forming one large median tetraploid nucleus between two smaller diploid nuclei. In 1933 D. C. Cooper examined the tapetal cells of forty-

three angiosperms and found three types of nuclear behavior as follows: (1) The tapetal cells do not divide at all and uninucleate cells are found throughout; (2) the nucleus divides once mitotically and the cells remain binucleate; (3) the nucleus may divide twice. If these divisions are regular four nuclei result in the same cell. If these divisions are incomplete and irregular uninucleate and binucleate cells may result.

While studying microsporogenesis in *Spinacia oleracea* a variety of atypical nuclear conditions were noted in the tapetal cells. A careful study of these cells showed that a type of division occurs in the tapetum of *Spinacia* which is new to tapetal cell literature. This process is called endomitosis. It was discovered in 1939 by Geitler in insect material and, as far as could be ascertained, this is the first time it has been demonstrated in plants. In this type of division there is a reduplication of the chromosomes, but no spindle is formed and there is no anaphase movement of the chromosomes. Throughout the whole process the nuclear membrane remains intact. The resulting nucleus and cell have twice the original number of chromosomes. Geitler divided endomitosis into four stages which he termed endoprophase, endometaphase, endoanaphase and endotelophase. Endoprophase is similar to the prophase stage of normal mitosis. The chromosomes contract by spiralization until a maximum degree of condensation is reached. This stage of maximum chromosome contraction is called endometaphase. The nuclear membrane remains intact. There is no spindle and the chromosomes do not congress upon an equatorial plate. After attaining their maximum contraction, the spindle attachment regions divide and the chromosomes separate slightly. This stage is called endoanaphase although there is no true anaphase movement of the chromosomes. During endotelophase the chromosomes undergo reversion to the resting stage condition. All the chromosomes remain in the same nucleus and

¹ Received for publication February 28, 1945.

The author wishes to thank Dr. C. A. Berger for his help and valuable suggestions throughout this work.

in this way a tetraploid nucleus is formed. In an endomitotic cycle, then, the chromosomes are reduplicated, the nuclear membrane does not break down, no spindle is formed and neither the nucleus nor the cell divides.

MATERIAL AND METHODS.—Anthers from the Old Dominion variety of *Spinacia oleracea* were used. The flower buds were fixed in a solution of three parts of absolute alcohol to one part of glacial acetic acid for twenty-four hours, after which they were transferred to Carnoy's fluid (6:3:1) for one hour. The anthers were then removed and placed on a slide in a drop of aceto-orcein stain. The contents of each anther were pressed out with needles through an opening made at one end. The stain was allowed to act for fifteen minutes, after which time the cells were smeared by applying slight pressure to the cover slip.

Smears stained by the Feulgen technique were also used. In this case, after fixation in the absolute alcohol-glacial acetic acid mixture, the anthers were dissected out and placed in a drop of forty-five per cent acetic acid. The cells were then pressed out and smeared by a slight pressure applied to the cover glass. The slides were then left for three hours in a moist chamber, after which the cover glasses were removed by inverting the slide in a dish containing one part of 95 per cent alcohol and one part of ten per cent acetic acid. The smears were then washed in water for ten minutes and hydrolyzed in normal hydrochloric acid at 60°C. for thirteen minutes. They were then placed in Feulgen stain for one hour. After staining they were washed in SO₂ water for ten minutes and dehydrated in a graded series of alcohols. From 95 per cent alcohol they were mounted in euparal.

Paraffin sections of whole buds cut at a thickness of ten microns were also used. For sections the buds were first placed in Carnoy's fluid for an hour to insure rapid penetration of the fixative. They were then placed in Craf fixative for twenty-four hours. The sections were stained with iron-alum hematoxylin and by the Feulgen technique. In the case of the Feulgen stain, twenty-two minutes hydrolysis was found to be necessary after fixation in Craf.

OBSERVATIONS.—In a young anther the tissue is undifferentiated and the cells undergo numerous mitotic divisions. Soon after these divisions cease the sporogenous tissue can be distinguished from the somatic tissue constituting the wall of the anther. When the pollen mother cells are in the resting stage the anther wall consists of four layers of cells, the epidermis, the endothecium, a single transition layer, and the innermost layer or tapetum. At this stage the tapetal cells are very small as compared to their size at maturity and the single nucleus is comparatively large. Young tapetal cells are square or oblong in shape, while the germ cells are polygonal. The nuclei of the germ cells are larger and less dense than the tapetal nuclei. Tapetal cells of *Spinacia* usually undergo only two divisions during the entire meiotic process and both of these divi-

sions occur while the nuclei of the pollen mother cells are in the zygotene synizesis stage of meiosis.

FIRST TAPETAL DIVISION.—The first division may be one of three types. In the first type the nucleus undergoes a normal mitotic division. There is no cell plate formed, however, and the two nuclei remain in the same cell (fig. 5). As a result a binucleate cell is formed, each nucleus being diploid. In the second type of division the chromosomes behave normally up to anaphase, when one or more of the

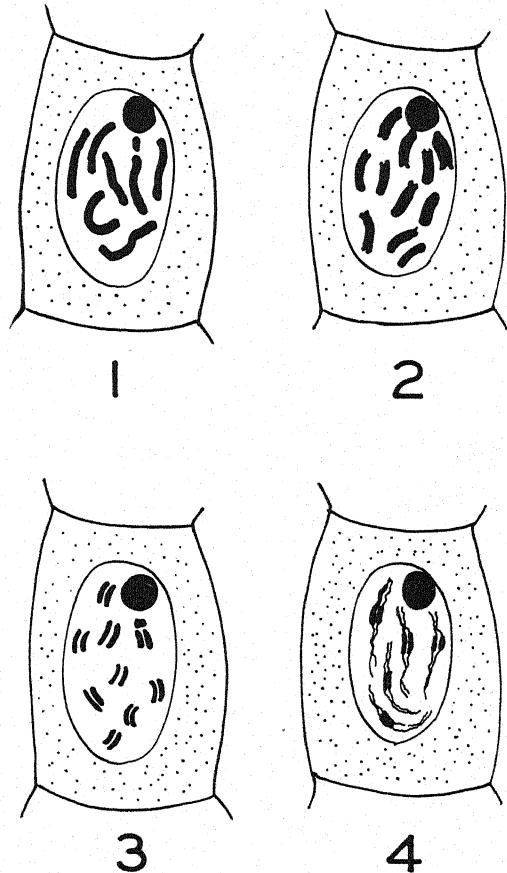


Fig. 1-4. Diagrams of the stages in endomitosis of diploid tapetal cells.—Fig. 1. Endoprophase.—Fig. 2. Endometaphase.—Fig. 3. Endoanaphase.—Fig. 4. Endotelophase.

chromosomes fail to separate, forming chromosomal bridges of the sticky type. These bridges persist through telophase and resting stage. As a result of this type of division a tetraploid cell is formed containing one dumb-bell-shaped nucleus. The strand connecting the two parts of this nucleus may be thick or thin depending on the number of chromosomal bridges involved (fig. 6 and 7).

The third type of first division is the endomitotic division. Once the idea of endomitosis is grasped, the four stages of the process described by Geitler (1939) and later by Painter and Reindorp (1939) can be clearly recognized in these tapetal cell divisions. Figures one to four illustrate these stages

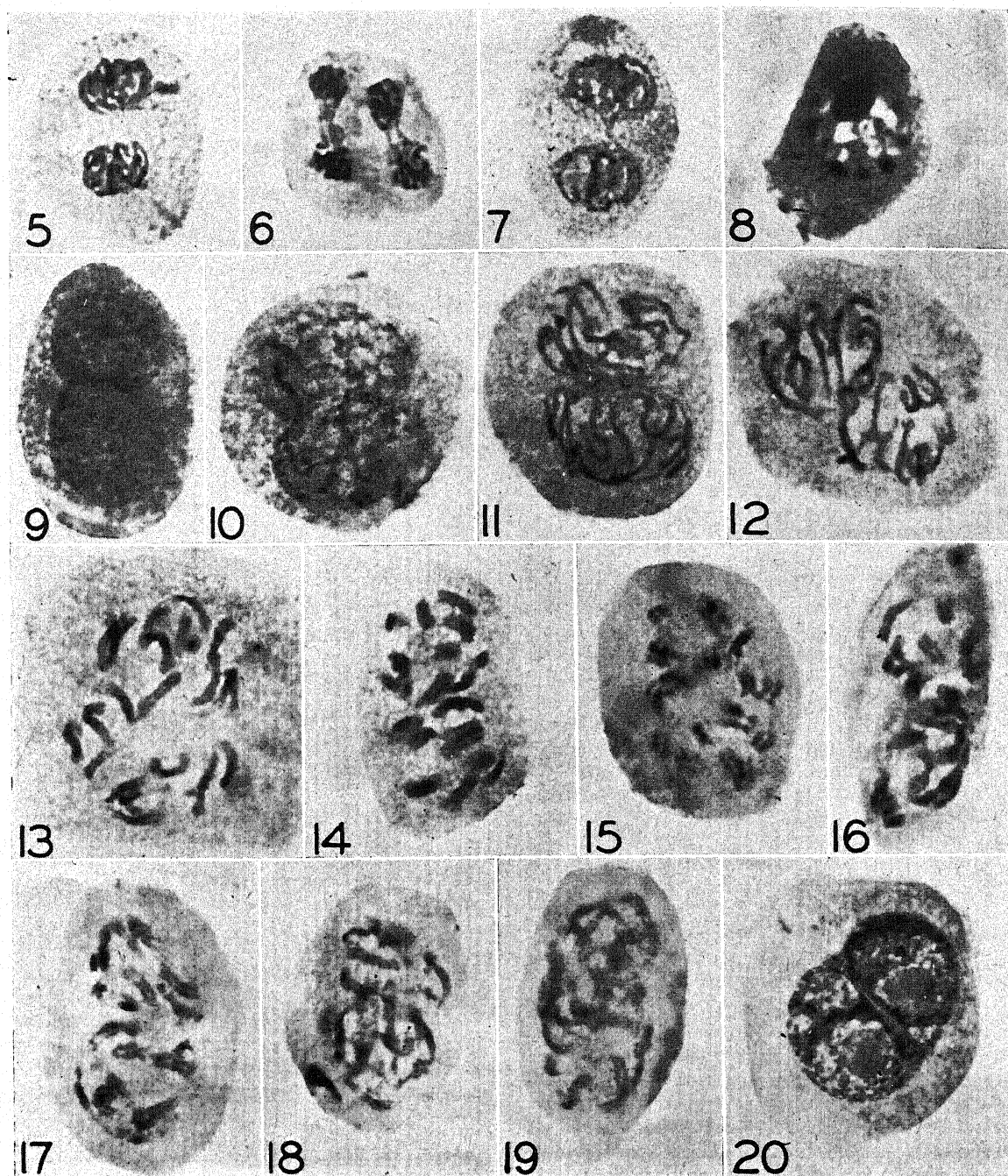


Fig. 5-20. Photomicrographs of tapetal cell divisions in *Spinacia oleracea*, $\times 1250$. All figures except figure 8 are of aceto-orcein smear preparations. Figure 8 is of sectioned material stained with iron-alum hematoxylin.—Fig. 5. Telophase nuclei without chromosomal bridges.—Fig. 6 and 7. Telophase nuclei showing chromosomal bridges.—Fig. 8. Endotelophase, showing despiralization proceeding from the ends of condensed chromosomes.—Fig. 9-20. Stages in endomitotic division of tetraploid dumb-bell-shaped nuclei.—Fig. 9. Resting stage.—Fig. 10-13. Successive stages in endoprophase.—Fig. 14. Endometaphase.—Fig. 15. Endoanaphase.—Fig. 16-19. Successive stages in endotelophase.—Fig. 20. Octoploid resting nucleus.

diagrammatically. During endoprophase (fig. 1) the chromosomes condense as in normal prophase and the two chromatids of each chromosome become evident. Contraction continues up to endometaphase

(fig. 2), the stage at which the chromosomes have reached their greatest degree of condensation. There is no spindle present and the chromosomes do not congress upon an equatorial plate. The nuclear

membrane is still intact and the nucleolus is clearly present. At endoanaphase the spindle attachment regions of the chromosomes divide and the chromatids separate slightly (fig. 3). This separation of chromatids without anaphase movement or nuclear division results in a visible doubling of the number of chromosomes. During endotelophase the chromosomes undergo a reversion to the resting stage condition (fig. 4). Despiralization begins at the ends of the chromosomes and in early endotelophase highly contracted chromosomes may be seen with fine threads emerging from their ends (fig. 4 and 8). The chromosomes become less chromatic and lose their sharp outlines. Reversion continues until a resting stage condition is reached. The resulting nucleus is tetraploid. Throughout the whole process the nucleolus is present and the nuclear membrane is intact. There is no division of the nucleus or of the cell.

These three types of the first tapetal cell division all occur while the sporocytes are in the zygotene synizesis stage. The nuclei resulting from this first division may remain in the resting stage throughout the rest of the meiotic process. Some of these cells, however, undergo a second division which likewise occurs while the sporocytes are in the zygotene stage and is always endomitotic.

THE SECOND DIVISION OF THE TAPETAL CELLS.—Second divisions have been found in binucleate cells and in tetraploid cells with interconnected, dumb-bell-shaped nuclei. In the first case both nuclei undergo endomitosis simultaneously, passing through endoprophase, endometaphase, endoanaphase and endotelophase as previously described. The resulting cell contains two tetraploid nuclei.

In the second case the interconnected nucleus likewise undergoes a typical endomitotic division including the four stages of Geitler. These endomitotic figures are striking in appearance. Figures nine to twenty are very clear and once a series of such stages is arranged in order, no doubt remains as to the type of division occurring. Many of these figures considered individually might well be taken for a stage in amitosis. A comparison of figures 11 to 13 with figures 16 to 19 will make clear the difference between endoprophase and endotelophase. The nucleolus is present at all stages of endomitosis.

All these divisions take place during the zygotene synizesis stage. During the remaining meiotic stages the tapetal cells remain in the resting condition but continue to increase in size. In a binucleate cell the enlarging nuclei may become closely appressed giving the illusion of a stage in nuclear fusion.

Disintegration of the tapetal cells begins when the sporocytes have divided to form the tetrad stage. The tapetal cells and their nuclei continue to increase in size. Dense chromatic material aggregates at the periphery of the nuclei. The nucleoli decrease in size and become less chromatic. The cytoplasm becomes less dense and appears to be somewhat

fibrous. The cells completely disappear by the time mature pollen grains are formed.

DISCUSSION.—In recent years it has become apparent that the production of polyploidy by chromosomal reduplication without nuclear division is a common occurrence in both plant and animal material. Endomitosis is only one of three known methods by which this can occur, although the term has been used to describe all three procedures in some of the recent cytological literature. Perhaps the clearest example of the first type is the production of highly polyploid cells in the ileum of mosquito larvae (Berger, 1937) by repeated reduplication of the chromosomes in the resting nucleus. The second way in which this polyploid condition may be brought about occurs in *Spinacia* and other polysomatic plants and consists in a double reproduction of the chromosomes in the resting nucleus as shown by Gentcheff and Gustafsson (1939) and by Berger (1941). In both of these methods the reduplication of the chromosomes occurs while the chromosomes are in the resting stage and there are no endoprophase, endometaphase, endoanaphase or endotelophase stages as described by Geitler (1939). It is not clear to which of these three types the salivary gland chromosomes of the Diptera belong, but they probably are a modification of type one. Types one and two result in polyploid cells which can and do undergo mitotic division as polyploid cells. From the evidence thus far published it would appear that cells which become polyploid by the third method, namely, endomitosis, are incapable of subsequent mitotic division. It is interesting to note that in *Spinacia* polyploidy arises in different ways in the polysomatic tissue of the root tip and in the tapetal cells.

A year before his discovery of endomitosis Geitler (1938) described a polyploid condition in several tissues of the plant *Sauromatum guttatum*. Grafl, a student of Geitler, continued the study of *Sauromatum* and stated (1939) that the polyploidy did not arise by endomitosis but in the same way that polysomaty originates in *Spinacia*. Jachimsky (1937) and Yampolsky (1937) also described polyploid conditions in the cells of various plants but one cannot be sure from these papers that the condition arose through endomitosis. Meyer (1925) found diploid cells in the tapetum of a young anther of *Leontodon officinale* and polyploid cells in the tapetum of mature anthers. Since he found no mitotic figures he assumed that the only possible explanation was the occurrence of an "Innere Teilung" or division of the chromosomes without a nuclear division. Since he does not describe or make any mention of stages corresponding to endoprophase, endometaphase, endoanaphase and endotelophase we must conclude that the process he assumes is not endomitosis as here described. Accordingly while there may be some doubt as to whether or not this is the first time endomitosis has been reported for plant tissue, it seems certain that it is the first description of this process in tapetal cells.

The presence of the nucleolus throughout the whole of the endomitotic cycle is of considerable interest since in *Spinacia* the nucleolus always disappears before metaphase. Its persistence through endomitosis may suggest a connection between the nucleolus and the formation of the spindle.

SUMMARY

The tapetal cells of *Spinacia* undergo two divisions during meiosis. Both of these divisions take place while the sporocytes are in the zygotene synizesis stage. The first division may be one of three types. Normal mitosis may take place but no cell plate is formed and a binucleate cell results. Secondly, the nucleus may undergo an abnormal mitosis due to the presence of sticky chromosomal bridges. As a result a uninucleate cell is formed with a dumb-bell-shaped nucleus. Thirdly, the cell may undergo a type of division new to tapetal cell cytology which is called endomitosis. The endomitotic cycle consists of endoprophase, endometaphase, endoanaphase and endotelophase. The chromosomes undergo contraction to the metaphase condition, the spindle attachment regions divide and the daughter

chromosomes separate slightly and revert to the resting stage condition. Throughout the whole process the nucleolus is present, the nuclear membrane remains intact, there is no spindle and consequently no true anaphase movement of the chromosomes. Thus all the chromosomes remain in the same nucleus, increasing the degree of polyploidy.

The resulting nuclei may remain in the resting condition or may undergo a second division. The second division is in all cases endomitotic. The cell resulting from this division is either a uninucleate octoploid cell, in which case the nucleus is dumb-bell-shaped, or a binucleate cell with two tetraploid nuclei.

It seems possible that endomitosis may not be peculiar to the tapetal cells of *Spinacia* but may have a wider application and may explain many of the cytological phenomena occurring in the tapetal cells of other plants, which up to now have been obscure.

BIOLOGICAL LABORATORY,
FORDHAM UNIVERSITY,
NEW YORK, NEW YORK

LITERATURE CITED

- BERGER, C. A. 1937. Additional evidence of repeated chromosome division without mitotic activity. *Amer. Nat.* 71: 187-190.
- . 1941. Reinvestigation of polysomaty in *Spinacia*. *Bot. Gazette* 102: 759-769.
- COOPER, D. C. 1933. Nuclear division in the tapetal cells of certain angiosperms. *Amer. Jour. Bot.* 20: 358-364.
- GEITLER, L. 1938. Über das Wachstum von Chromozentrenkerne und zweierlei Heterochromatin bei Blütenpflanzen. *Zeitschr. f. Zellforsch.* 28: 133-153.
- . 1939. Die Entstehung der polyploiden Somakerne der Heteropteren durch Chromosomenteilung ohne Kernteilung. *Chromosoma* 1: 1-22.
- GENTCHEFF, G., AND A. GUSTAFSSON. 1939. The double chromosome reproduction in *Spinacia* and its causes. *Hereditas* 25: 349-358.
- GRAFL, I. 1939. Kernwachstum durch Chromosomenvermehrung als regelmässiger Vorgang bei der pflanzlichen Gewebedifferenzierung. *Chromosoma* 1: 265-275.
- JACHIMSKY, H. 1937. Zur Zytologie der Riesen-Antipodenkerne. *Planta* 26: 608-613.
- MEYER, K. 1925. Über die Entwicklung des Pollens bei *Leontodon autumnalis* L. *Ber. Deutsch. Bot. Ges.* 43: 108-114.
- PAINTER, T. S., AND E. REINDORF. 1939. Endomitosis in the nurse cells of the ovary of *Drosophila melanogaster*. *Chromosoma* 1: 276-283.
- SMITH, F. H. 1933. Nuclear divisions in the tapetal cells of *Galtonia candicans*. *Amer. Jour. Bot.* 20: 341-347.
- STRASBURGER, E. 1882. Ueber den Theilungsvorgang der Zellkerne und das Verhältniss der Kerntheilung zur Zelltheilung. *Arch. Mikrosk. Anat.* 21: 476-590.
- TISCHLER, G. 1906. Über die Entwicklung des Pollens und der Tapetenzellen bei *Ribes*-Hybriden. *Jahrb. Wiss. Bot.* 42: 545-578.
- YAMPOLSKY, C. 1937. The cytology of the ovarian trichomes of *Mercurialis annua*. *Cytologia* 8: 208-219.

A REMARKABLE TREE-FALL AND AN UNUSUAL TYPE OF GRAFT-UNION FAILURE¹

Arthur J. Eames and L. G. Cox

IN NOVEMBER, 1941, Cornell University received from Norwich, New York, the basal end of the trunk of a white fir tree (*Abies concolor* L.) that had fallen "in a mysterious manner." The tree, an ornamental specimen grown in a cemetery, was about 40 years old, 25 feet tall, and 12 inches in diameter at the base. It was found fallen, separated cleanly from the stump just below the ground level. The break was nearly transverse and remarkably smooth (fig. 1, 3), suggesting to local people that it had been sawed down or that some animal had gnawed it down.

It seemed probable that the fall was the result of a break in tissues along a line of incomplete graft union and members of the staffs of the Departments of Forestry and Botany went to Norwich and dug up the stump for further evidence of the cause of the fall. When the basal section of the trunk is fitted to the stump, a transverse furrow is evident in the bark along the line of break (fig. 2) and the trunk just above the line of break is seen to be slightly greater in diameter than the stump. The undulate surfaces of separation show, on opposite sides of the pith, a projecting hump and a concavity (fig. 1, 7). Both of these facts supported the view that the fall was the result of graft-union failure. Anatomical study of the region of the break confirmed this view and demonstrated the presence of a possibly new type of failure of anatomical union of stock and scion.

Slight color differences of the inner bark indicated the probability that a different species was used as stock but these could not be used in identification, and wood characters also proved of no value. Bailey (1935) states that varieties and forms of firs are grafted on seedlings of the particular species, if these are available; if not, on those of *Abies balsamea* (L.) Mill., *A. concolor* Lindl. and Gord., and *A. alba* Mill. (= *A. Picea* Lindl.; *A. pectinata* DC.). Though foliage forms of white fir are grafted on seedlings of the same species, it seems unlikely that the stock in this tree was of that species because of the differences in bark color, rate of growth, and a probable incompatibility. It is unlikely that the balsam fir was used because of the lack of vigor of growth of this species. That the European white fir was used is possible since at the time this tree was planted—about 1900—much grafted fir nursery stock probably came from Holland.

Detailed anatomical study disclosed the story of the method of grafting used and the progress of growth to the fall of the tree. Perfectly preserved in the heart of the tree is the region of grafting, with the cut surfaces of stock and scion, the thread which held stock and scion together, and the callus

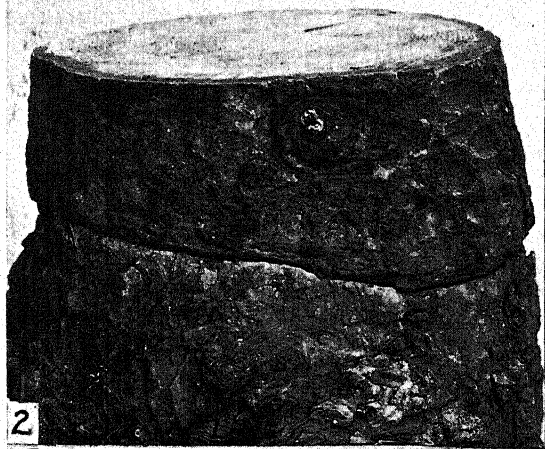
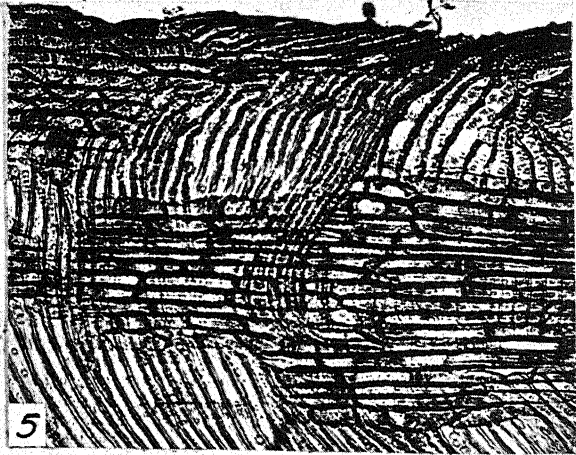
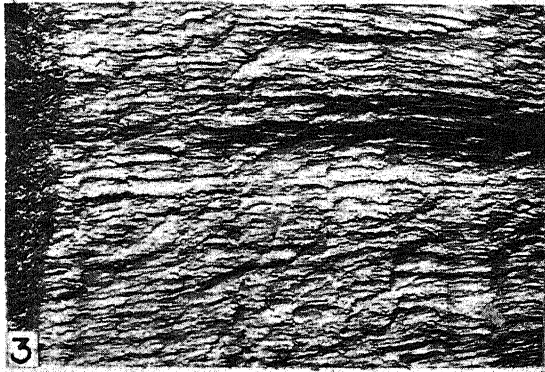
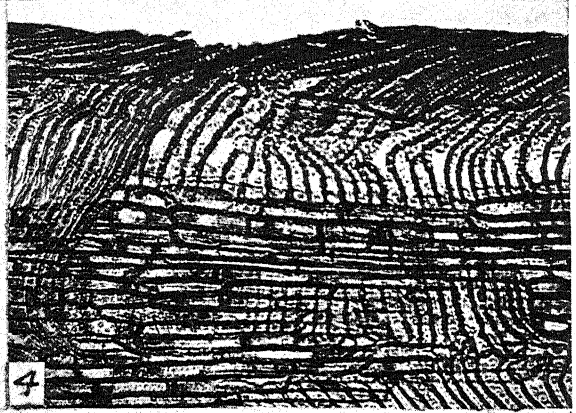
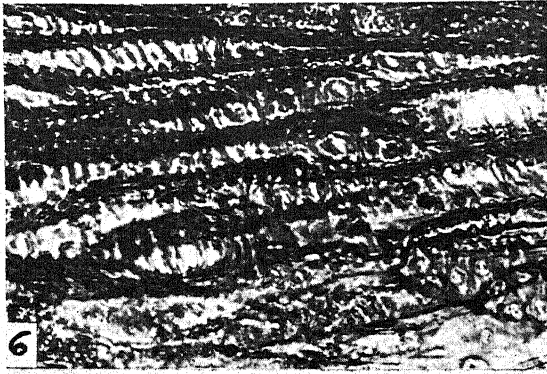
tissue which closed the wound areas (fig. 8). The surfaces of rupture and the furrow in the bark show the position of the cambium layers of both stock and scion (fig. 7, 14–16) and the structural basis for the extraordinary break in the tree.

The grafting method used was that known as "lateral," in which the scion was applied to the side of the stock. In preparation of the stock for grafting the cut was made extremely deep, well beyond the pith (fig. 10) so that less than half of the stem remained at that point. The position of the pith (fig. 8) determines the depth of the cut, as does also the absence of the pith in a transverse cut higher up (fig. 9). This doubtless accidental condition may have been in part the cause of the graft-union failure. The scion, applied obliquely to the cut, was tied in by a linen thread (about 0.3 mm. in diam.) (fig. 11) which is preserved in position, overgrown by the later-formed xylem of stock and scion (fig. 8, 12). The lower part of the cut-surface of the stock, resin-coated, is clearly seen in the dissection (fig. 8), as is also the short oblique cut by which the top of the stock seedling was later removed (fig. 8, 12). Overgrowth by callus and xylem and phloem took place rapidly on these surfaces, both inward from the sides and downward over the top (fig. 8, 9, 12, 13). Some bark remains on this overgrowth adjacent to the scion (fig. 8). Overgrowth was so extensive and continued so long—three to six years—that it is clear that there was no close contact of the upper cut-surfaces of stock and scion; the tying-in of the scion was defective above, and the two plant-tops apparently stood apart, held together tightly only at the base of the union (fig. 11, 12).

Callus development rapidly covered most of the wound surface of the stock, leaving contact with only the very base of the scion. Similarly most of the cut surface of the scion became callused. From the beginning, therefore, there was contact of vascular tissues over only a small part of the possible surface, and the cambium margins failed to unite. The failure of cambium union was probably due to incompatibility of stock and scion but may have been in part because of the early establishment on both stock and scion of callus that almost completely enclosed each wound area independently.

The margins of the cambium cylinders lay close together but remained unfused. The position of the ends of the cylinders is evident from the form of the annual rings (fig. 16). The in-turned ends of the cambia formed tracheids which, similarly, were bent inward at right angles or lay horizontally in the tree (fig. 4, 5). Thus there was no interlocking of the cells of the stock and scion, and horizontally lying vascular cells separated the stump of the tree from the trunk (fig. 4, 5, 14, 15). Contact between

¹ Received for publication February 26, 1945.



tracheids and sieve tubes of the stock and scion was intimate, but lateral; no parenchyma or "wound tissue" lay between these cells. The transversely oriented vascular cells apparently functioned normally in conduction between stock and scion because the tree grew well for forty years.

That a large and well-grown tree could stand so long with a layer of transversely oriented tracheids across the base of trunk is extraordinary because such a layer must represent a region of extreme mechanical weakness. Without interlocking of tracheids the tree must have been kept from falling only by intercellular substance. Only remarkable protective conditions could have saved so weak a tree from early destruction. The tree was healthy, and, according to the statement of the superintendent of the cemetery, had grown normally. It had been protected, however, from wind by an unbroken circular hedge of red cedar and arbor vitae which was about 15 feet tall at the time the tree fell. Probably the fallen tree had only recently sufficiently overtopped this shelter to expose a considerable portion of its top to the wind. Further, the cemetery is so located in a valley as to be exposed to strong winds only from the southeast. Such winds had blown briefly about the time the tree fell. (The exact time of the fall is unknown.)

The transversely placed cells are chiefly those of the stump (fig. 14); the ends of its annual-ring cylinders turn inward more sharply than do those of the trunk and taper out as thin flat wedges (fig. 8, 14, 15). The margins of the annual-ring cylinders of the trunk are rounded and lack tapering edges (fig. 8, 14, 15). When the tree fell, the break followed closely the line between the tissues of stock and scion except in a few places where fragments of the trunk tissues remained attached to the stump, especially in the outermost annual ring (fig. 15). Here, because of the greater diameter of the trunk, the xylem of the last annual ring lay upon the inner phloem of the stump, its convex rim matching the concave rim of the phloem cylinder, and the break came through the projecting rim of trunk xylem in some small areas. The surface of the break is remarkably smooth because of the transverse position, compactness, and strong lignification of the cells of the region (fig. 3, 4, 5). The prominent hump and hollow of the central region (fig. 1, 7) represent the side-by-side tissues of stock and scion in the early years; later these were "smoothed out" and the tissues of scion and stock stood only above and below (fig. 8, 16).

The transversely placed tracheids of the stump show remarkable structure (fig. 6). They are short and of small diameter, with the walls covered with

crowded bordered pits. The thick, heavily lignified secondary walls have closely placed transverse ridges. In appearance these ridges suggest the bars of scalariform and close-spiral protoxylem tracheids. In structure they resemble the "stretched" tracheids of the leaf traces of conifers where the trace is ruptured and added to as the evergreen leaf is pushed outward by the increase in diameter of the stem. In these leaf traces, however, the tracheids become delignified and ruptured. Aside from the presence of the transverse ridges, this thin layer of wood resembles the compression wood of conifers. It lay in a position where, as it matured, it was subjected to extreme compression, especially if the tree rocked when swayed by the wind. The unusual wall thickenings represent, perhaps, strengthening areas related to compression.

Graft-union failure of structural type where no parenchymatous cells lie between the vascular tissues of scion and stock appears to be unknown. Descriptions in literature of the anatomy of graft-unions where incompatibility exists are few and none covering conditions in conifers has been found. All note the presence of parenchyma-like tissues along the surface of union, with cambium union delayed or never attained.

Goeppert (1874) reported a parenchymatous union between stock and scion in interspecific grafts of *Sorbus* where the connecting tissue appeared to the naked eye as a "thin greenish stripe." This layer persisted through the first annual ring, and only in the second year did cambium continuity develop. Traces of the parenchymatous union-tissue could be found in tangential sections long after this could be seen in transverse sections.

Waugh (1904) described a three-year-old graft union of pear on quince that was defective because "loose roundish cells" (probably parenchyma) formed a thin division wall between the tissues of stock and scion.

Proebsting (1926) found, instead of the layer of parenchyma between stock and scion, a sheet of bark tissue. This bark could be divided roughly into a middle layer of corky nature with, on each side, a layer characteristic of the bark of the species. The layers extended nearly to the point at which the cambium layers of stock and scion were placed in contact when the graft was made. Proebsting assumed that, after the inception of growth, the part of the cambium that lay at the line of union ceased to function, and as growth continued above and below this region, a layer of bark was laid down by the cambium of both stock and scion. This condition was observed in one-year-old budded and grafted material of *Pyrus Malus* on *P. serotina*,

Fig. 1-6.—Fig. 1. Broken surfaces of stump and trunk of 40-year-old *Abies concolor* fallen through graft-union failure. $\times \frac{1}{8}$.—Fig. 2. Stump and base of trunk showing furrow in bark and difference in diameter above and below. $\times \frac{1}{8}$.—Fig. 3. Portion of broken surface of trunk showing transversely lying wood tissues. $\times 3$.—Fig. 4. Radial section of xylem of trunk at line of break, showing form of annual rings and position of tracheids. $\times 180$.—Fig. 5. Radial section of xylem of stump at line of break, showing position of tracheids forming transverse plate and lack of interlocking with xylem of trunk. $\times 180$.—Fig. 6. Tracheids of the transverse plate with heavy transverse ridges of secondary wall. $\times 415$.

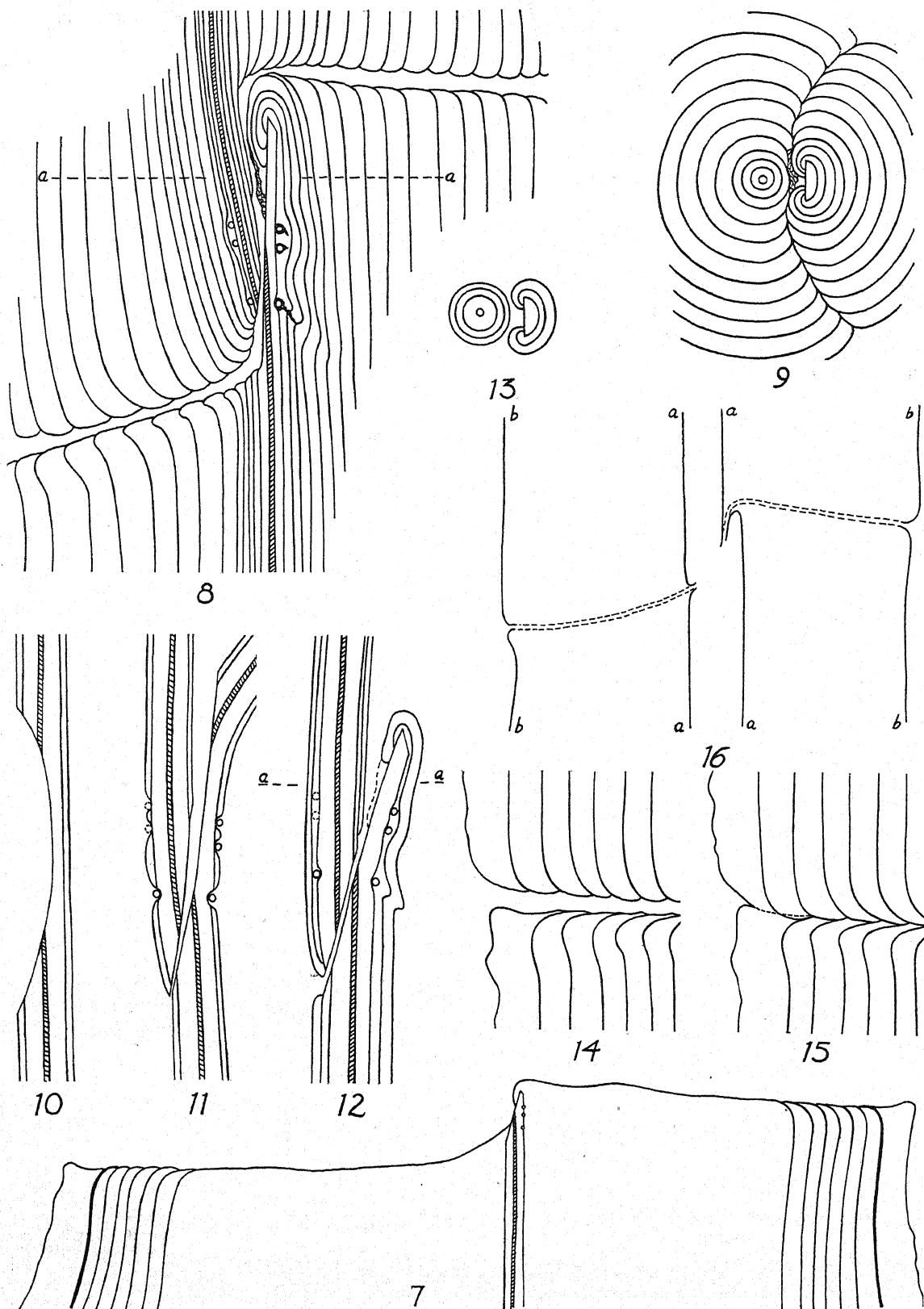


Fig. 7-16.—Diagrams showing anatomy of region of graft-union failure.—Fig. 7. Profile of surface of stump in plane of grafting, showing first annual ring with cut surfaces for grafting and removal of top of seedling, thread

P. Malus on *P. communis*, *P. communis* on *P. Malus*, *P. communis* (var. Bartlett) on *Cydonia oblonga*, and *C. oblonga* on *P. serotina*.

The several types of structural defects of graft union were summarized by Proebsting (1928) as (1) the formation of a double layer of bark accompanied by a considerable amount of wound parenchyma between stock and scion; (2) the formation of wound parenchyma by the cambium of stock or scion, or by both cambia, at the line of union, with or without distortion of the adjacent vascular tissue; (3) distortion of the vascular tissue which in mild cases may not cause mechanical weaknesses or functional disturbances, but may cause difficulties in translocation when the distortion is so great that whorls and loops of vessels are formed in both longitudinal and transverse planes; and (4) degeneration at the line of union, with formation of gummy masses, of all the xylem between the rays.

Bradford and Sitton (1929), after a study of defective graft unions, concluded that uncongenial grafts, as represented by pear on apple, or pear on quince, differed from congenial grafts principally in a failure to maintain cambium continuity, the break coming apparently at the end of the growing season. With continuing growth of stock and scion, this cambium break leaves a zone of parenchymatous tissue and both xylem and phloem are discontinuous.

SUMMARY

A forty-year-old, cultivated white fir fell because of a graft-union failure which resulted in a clean transverse break at ground level. Anatomical study demonstrates the type of grafting used and the structural conditions which developed as the tree grew. One-year-old seedling stock was used, the cut for the lateral application of the scion was made too deeply—beyond the pith—and the scion was apparently tied in too loosely. Incompatibility of

stock and scion were doubtless responsible for a failure of the cambium layers to unite, though the structural conditions of the poor graft may have also been in part responsible. The stock was doubtless not the same species as the scion though it has not been possible to identify this from wood or bark structure.

The edges of the cambium cylinders of scion and stock failed to unite but turned inward at the line of union. From these marginal parts a transverse plate of vascular tissue was built up across the trunk of the tree wherein the ends of the tracheids and sieve tubes lay horizontally. No parenchyma was formed between the two uncongenial tissue masses, a condition apparently not heretofore described in graft-union failure. There was no interlocking of the tracheids of stock and scion and the horizontal plate formed a zone of great mechanical weakness. The protection of the tree from wind by a high hedge doubtless preserved it from destruction for many years.

DEPARTMENT OF BOTANY,
CORNELL UNIVERSITY,
ITHACA, NEW YORK

LITERATURE CITED

- BAILEY, L. H. 1935. The standard cyclopedia of horticulture. Macmillan Co.
BRADFORD, F. C., AND B. G. SITTON. 1929. Defective graft unions in the apple and in the pear. Michigan State Tech. Bull. 99.
GOEPPERT, H. R. 1874. Ueber innere Vorgänge bei dem Veredeln der Bäume und Sträucher. pp. 1-32. Cassel.
PROEBSTING, E. L. 1926. Structural weaknesses in interspecific grafts of *Pyrus*. Bot. Gaz. 82: 336-338.
———. 1928. Further observations on structural defects of the graft union. Bot. Gaz. 86: 82-92.
WAUGH, F. A. 1904. The graft union. Massachusetts (Hatch) Agric. Exper. Sta. Tech. Bull. 2.

for tying, and form of later annual rings. $\times \frac{1}{2}$.—Fig. 8. Detail of center region, radial section, with tissues separated as by the break, showing relation of stock and scion, deep cut of stock, overgrowth on cut surfaces, form of successive annual rings, included bark about thread and on cut surface of stock.—Fig. 9. Cross section of center of region shown in figure 8, in plane *a-a*.—Fig. 10. Stock as cut deeply for grafting.—Fig. 11. Stock and scion, in grafting position.—Fig. 12. Stock and scion two years later (separated), bark omitted.—Fig. 13. Cross section in plane *a-a* of figure 12.—Fig. 14, 15. Form of edges of annual rings on line of contact: figure 14, stock (below) and scion broken apart; figure 15, stock and scion united, dotted line indicating position of occasional rupture through tissues of scion.—Fig. 16. Form of cambium cylinders two years after grafting (*a-a*) and at tree fall (*b-b*).

A PHYSIOLOGICAL SEPARATION OF TWO FACTORS NECESSARY FOR THE FORMATION OF ROOTS ON CUTTINGS¹

J. van Overbeek and Luis E. Gregory

DESPITE THE fact that in many instances auxins² and allied substances have been successfully employed in the rooting of cuttings, many plants still fail to respond to this or to any other known treatment. Among the tropical plants in which cuttings are difficult to root, there are many of considerable economic value, such as the avocado, cinchona, hevea, and mango. It is noteworthy that many of the species which are difficult to root are woody. It seemed desirable, therefore, to make a study of the physiology of the rooting process of a woody plant. Hibiscus was selected for this purpose, and the first series of experiments is reported here. Many hibiscus varieties grow abundantly in the tropics and are capable of providing the experimenter throughout the year with an almost unlimited supply of relatively uniform material.

Cuttings of most hibiscus varieties may be rooted with ease. However, in the course of our work, a form was found which is difficult to root. This form, which is white-flowered and known as Ruth Wilcox, is so difficult to root that in practice it is propagated only by grafting on established root stocks of the easy-rooting red species. The red-flowered and white-flowered hibiscus are closely related, but they differ considerably in ease of rooting. It seemed to us, therefore, that the white form might be a suitable object for a study designed to learn more about the reasons why cuttings of certain plants are difficult to root.

The present experiments indicate that the white form fails to root because two essential factors are deficient in its cuttings. Both of these have to be present in order to make the cuttings root. One factor is auxin, while the other one is a factor or combination of factors found to be present in the leafy shoots of the red species. Auxin can be supplied by treatment of the base of the cutting. It was found that the second factor, or factors, could be transmitted to the cuttings of the white form by grafting a leafy shoot of the red species on it as a donor. With both the auxin and the unknown factor supplied, the white form rooted abundantly.

The present article stresses the physiological separation of the auxin and the above mentioned additional factor. A further analysis of the reasons which make the white form difficult to root will be published elsewhere (Gregory and van Overbeek, 1945). Experiments designed to identify the chemical nature of the root-inducing factors of leafy shoots are in progress.

MATERIAL AND METHODS.—*Hibiscus rosa-sinensis* L. and a *Hibiscus* hybrid which appears to be the form *Ruth Wilcox* were used. *H. rosa-sinensis* L. is the common red hibiscus mostly used for hedges.

¹ Received for publication January 12, 1945.

² For terminology see van Overbeek (1944).

It roots very well, and it is almost exclusively propagated by means of cuttings. Terminal branches having from four to six leaves, unless otherwise stated, were used both as scions in the grafting experiments and as cuttings. These branches were mostly one year old and were cut from a hedge of old plants. The form *Ruth Wilcox* is a white-flowered hybrid. It originated from a cross of the variety *May Damon* × *Knudsen White* (Wilcox and Holt, 1913). *Knudsen White* is a form of *H. weimeae*. It is difficult to propagate by cuttings, especially if young terminal branches are used. In practice it is propagated mainly by grafting on root stocks of the red *Hibiscus rosa-sinensis* L. The cuttings used in these experiments were "hardwood" cuttings about 12 cm. long and 1 cm. in diameter.

Graft technique.—Two grafting methods (Yerkes, 1932) were tried, both of which gave equally satisfactory results. The bark graft was most extensively used. The scions were grafted immediately on the freshly made cuttings as a stock. In some experiments the bottle graft method was used, which is a modified whip graft in which the whip of the scion is extended and put into a vial of water. This has a theoretical advantage in that it keeps the scion provided with water during the period before the union has established itself.

Auxin treatments.—Indolebutyric acid in a concentration of 2 mg/cc was employed and applied by the alcohol dip method (Hitchcock and Zimmerman, 1940; Cooper, 1944). In preliminary experiments it was found that approximately 2 mg/cc of indolebutyric acid is the most satisfactory concentration for hibiscus. Concentrations as high as 10 mg/cc can be used, but the base of the cutting will die while roots are formed just above the damaged portion of the stem. Auxin treatments in grafting experiments were usually made immediately after the grafts were made. In some experiments, auxin treatments were given a few weeks after the grafts were made and the union had been established.

After treatment, the cuttings were put to root in the sand of the propagating frame. This is a brick structure provided with a shaded glass roof and roof spray. The average temperature of the sand was found to be 25°C., and the air humidity was kept high. Some experiments were conducted in an electrically heated hot bed with a temperature of 31°C. A detailed description of both structures has been reported elsewhere (van Overbeek, Tió, Gregory and Vázquez, 1943-44).

INTERACTION OF AUXIN AND OTHER ROOT FORMING FACTORS.—Terminal leafy shoots of the white form can not be used satisfactorily for rooting experiments, since they often disintegrate in a few days. However, it was found that during the winter season these cuttings remained in better condition than

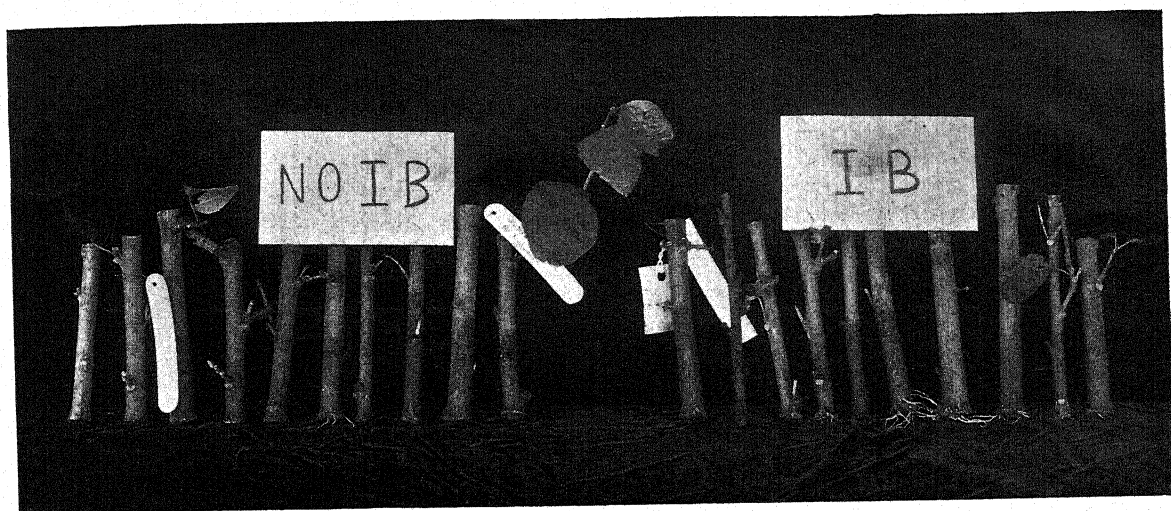


Fig. 1-2.—Fig. 1. Cuttings of white hibiscus with and without auxin treatment. The treatment (IB) was given 26 days after the cuttings were made, in order to make the experiment comparable to those of figure 2 of which they are a part. Photographs made 19 days after IB treatment.—Fig. 2. Shoots of red hibiscus (R) grafted on cuttings of white hibiscus (W). The base of W of one series was treated with 2 mg/cc of indolebutyric acid (IB) by the alcohol dip method, 26 days after the grafts were made. The photograph was made 19 days later. The controls, white hibiscus without grafts but with IB, are shown in figure 1. Further data in table 1. Rooting takes place only as a result of the combined action of auxin and the red hibiscus scion.

during the summer. "Hardwood" cuttings of the white form were, therefore, used exclusively. Without treatment these cuttings root poorly, as is shown in the left hand portion of figure 1 and in table 1. Auxin treatment of the base of these cuttings does not materially increase the number of roots (figure 1, right, and table 1). This failure of the cuttings of the white form to respond to auxin may be due to several causes. One of these is the possibility that root-forming factors, other than auxin, may be deficient in the white form. Since the red species responds well to auxin, with an increase in the number of roots formed (figure 4 comparing the "IB" with the "no IB" curves), it was concluded that in the leafy shoots of the red species auxin

limits the root formation while other necessary factors are present in relative abundance. This consideration led to attempts to transmit these factors from the red into the white cuttings by means of grafting. Grafting without additional auxin treatment failed to induce roots in the white cuttings (fig. 2 left). When, however, the base of the white cuttings on which red shoots were grafted, was treated with auxin, an abundant root formation was observed (fig. 2, right).

This experiment demonstrates that in order to force cuttings of the white hibiscus to produce roots, both auxin and another factor coming from the leafy shoot of red hibiscus are necessary. Both factors have to act coordinately, since each by itself is in-



Fig. 3. Effect of the leaves of R on root formation in auxin-treated R/W cuttings. Photographed 29 days after the grafts were made and the base of W was treated with 2 mg/cc of indolebutyric acid by the alcohol dip method.

TABLE 1. *Effect of indolebutyric acid (IB) and the scions of red hibiscus (R) on the root formation of white hibiscus cuttings (W).* (See photographs fig. 1 and 2.) In A, R grafted on W by bark grafting. IB treatments of the base of W 26 days after the grafts and cuttings were made. Observations 19 days later. Averages of 10 cuttings. In B, R grafted on W by bottle grafting. IB treatment of the base of W made at the time the grafts were made. Observations 19 days later. Averages of 10 cuttings. In C, experiments as under A, except that the bark of the upper part of W was half or completely girdled (see photographs fig. 5 and 6).

Type of cutting	IB treatment	Weight of roots per cutting, mg.	Number of roots per cutting
A			
W	—	3 ± 3	0.3 ± 0.1
W	+	10 ± 5	3.7 ± 1.4
R/W	—	61 ± 30	1.1 ± 0.7
R/W	+	984 ± 140	32.6 ± 3.9
B			
R/W	—	0.7 ± 0.5
R/W	+	25.0 ± 5.5
C			
R/W, ½ girdle.....	—	130 ± 58	1.8 ± 0.5
R/W, ½ girdle.....	+	400 ± 140	24.4 ± 3.2
R/W, complete girdle	—	0	0
R/W, complete girdle	+	0	0

effective. This grafting experiment has been repeated ten times, using a total of 600 plants, and always with the same results.

LEAVES AS A SOURCE OF ROOT-FORMING FACTORS.—After the root-inducing properties of the leafy shoots of red hibiscus were ascertained, the next

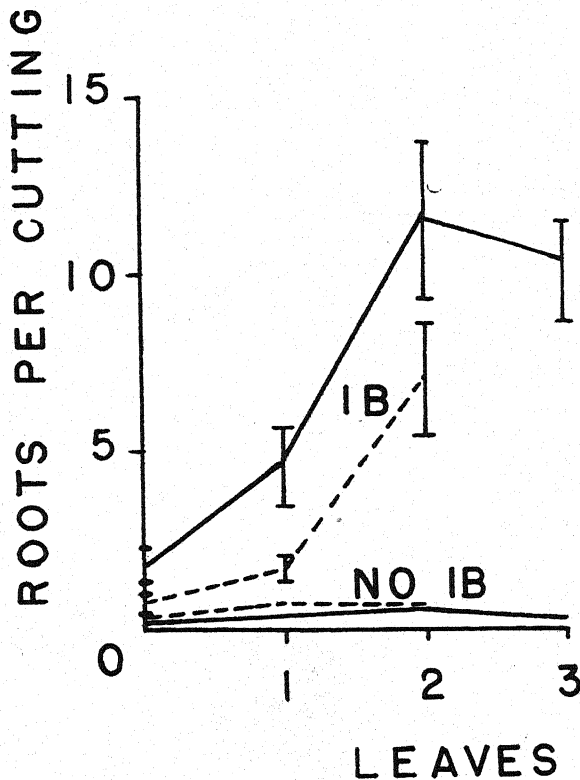


Fig. 4. Influence of leaves on the root formation of red hibiscus cuttings. Terminal and axillary buds were removed. The vertical lines indicate the standard error. Averages of 20 cuttings. "IB": base of cuttings treated with 2 mg/cc of indolebutyric acid by the alcohol dip method. Solid curves: sand of hot bed 31°C., observations after 11 days. Broken curves: sand of propagating frame 25°C., observations after 14 days. The experiment shows that leaves increase the root formation only in the presence of auxin.

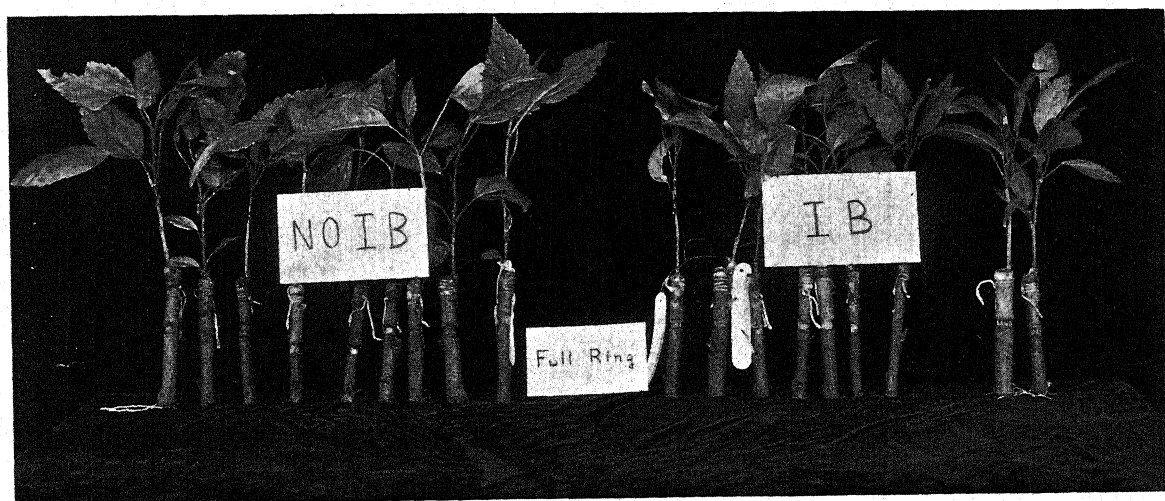


Fig. 5-6.—Fig. 5. Experiment showing that in R/W cuttings a half-girdle through the bark of W, made just below the scion, will not block the downward passage of the root-forming substance. Its movement is not strictly rectilinear. Experimental set-up as in figures 1 and 2. Additional data in table 1.—Fig. 6. Experiment showing that in R/W cuttings a complete girdle of the bark of W will block the downward movement of root-forming substances coming from the scion. Experimental set-up as in figures 1 and 2. Additional data in table 1. Wound regenerated in rooted cuttings on extreme right and left.

consideration which arose was to determine which part of the shoot is responsible for this effect. Grafts of red hibiscus shoots on white stock were used. As is shown in table 2 and figure 3, the leaves appear to be the principal source of the root-forming factor, although buds may show some activity. In these experiments the cuttings were treated with auxin in order to make sure that auxin did not limit root formation. The effect of the leaves, therefore, may be attributed to a root-forming factor other than auxin.

Cuttings of the red species, not grafted on white, also clearly showed the root-forming influence of leaves. This is presented in figure 4, which shows that only in the presence of auxin (IB curves) do leaves increase root formation. This is another dem-

onstration of the necessity of auxin plus a factor from the leaves in the process of root formation.

TRANSPORT OF THE ROOT-FORMING FACTOR.—When in grafting experiments a ring of bark was removed from the white cutting (complete girdle), the flow of root-forming substances coming from the red hibiscus shoots was completely intercepted, showing that it moved through the bark. The results of this experiment are shown in figure 6 and table 1. Only when the girdle was bridged by wound tissue were roots formed again at the base of the cutting. This was the case with the cuttings shown on the extreme left and on the extreme right of figure 6.

The root-forming factor of the red hibiscus shoots will also move sideways throughout the bark. This

TABLE 2. Influence of the leaves of the scion on the rooting of the stock in R/W hibiscus cuttings. Total number of cuttings, 30 in each series. Averages are based on cuttings which actually rooted (see fig. 3). Base of cuttings treated with indolebutyric acid at the time the grafts were made. Observations 29 days later.

	Leaves removed	Leaves present	Difference
Number of roots per cutting.....	5.5 ± 1.4 (n = 18)	18.8 ± 4.7 (n = 20)	13.3 ± 4.9
Weight of roots per cutting, mg....	38 ± 8 (n = 18)	243 ± 44 (n = 20)	205 ± 45
Percentage of cuttings which failed to root	40	33	

was demonstrated by removing a portion of the bark ($\frac{1}{2}$ girdle) of the white cuttings just below the place where the red shoot was grafted on, leaving the opposite part of the bark intact. Figure 5 and table 1 show that a half girdle did not materially interfere with the transport of the root-forming substances.

DISCUSSION.—In the analysis of those cuttings which are difficult to root it is not possible to say *a priori* whether the failure to root is due to lack of root-forming substances—nutritional or hormonal or both—or to certain inherent morphological characteristics of the cuttings (Thimann and Delisle, 1939). The fact that in our experiment the difficult-to-root white hibiscus form was, by means of a special procedure, successfully rooted, eliminates the possibility that failure to root in this case is due to morphological characteristics.

The experiments discussed above (fig. 1 and 2) allow the following conclusions to be drawn: (1) White hibiscus cuttings are deficient in auxin, which is one of the reasons for their failure to root; (2) white hibiscus cuttings are also deficient in a factor that may be provided by red hibiscus shoots; (3) the substance of the red hibiscus cuttings is probably not an auxin, since indolebutyric acid is effective only when shoots of red hibiscus are present; (4) the data of figures 3 and 4 show that the leaves of red hibiscus are an important source of this root-forming substance.

Went (1929) found that in *acalypha* the root-forming effect of the buds is greater than that of the leaves. These results were confirmed, as shown in table 3 in the third column. However, in auxin-treated *acalypha* cuttings the leaves are of major importance (table 3, column 4), as in hibiscus. These results suggest that the root-forming influence of *acalypha* buds is an auxin effect.

A beneficial effect of leaves on root formation of cuttings has been recognized for several years (for literature see Went and Thimann, 1937). However, Cooper (1935) and Cooper and Manton (1937) were the first to study the effect of leaves on cuttings which had received an optimum amount of auxin. It is by such experiments that conclusions can be drawn as to the existence of root-forming factors other than auxin, which arise in the leaf.

Our grafting experiments also demonstrate the presence of root-forming factors other than auxin

in the leaves of red hibiscus. Although Cooper (1938) and Went (1938, 1939) postulate the existence of hormonal root-forming substances other than auxin, the possibility is not excluded that the root-forming factor (or factor complex) of red hibiscus leaves may be (in part at least) nutritional. Thus, Beal (1940) reported that root formation of small isolated sections of bean stems will take place in the presence of sucrose and KNO_3 . In addition, Thimann and Poutasse (1941), also working with beans, reported a considerable increase in the number of roots as a result of treatments with KNO_3 . Investigations are in progress in an attempt to learn more about the chemical nature of the root-forming factors of red hibiscus leaves.

TABLE 3. Effect of the buds and the leaves on the rooting of *acalypha* cuttings, with and without treatment with 2 mg/cc of indolebutyric acid (IB) by the alcohol dip method, at the base of the cutting. If leaves were present, their number was 5. Averages of 20 cuttings in each series. Age of cuttings 10 days.

Leaves	Buds	Number of roots per cutting	
		Without IB	With IB
—	—	0.1 ± 0.2	13.4 ± 4.0
—	+	4.6 ± 2.0	9.6 ± 5.8
+	—	0.8 ± 0.4	40.0 ± 8.0

In the ringing experiments mentioned above (fig. 5), no evidence was found for a purely rectilinear basipetal transport of the root-forming substance of the leaves of red hibiscus, such as was found by Cooper (1936) for indoleacetic acid in the bark of lemon cuttings, and Went (1933), for a root-forming substance in *acalypha*, which might have been auxin also (see above).

The technique of using shoots of easy-rooting varieties as donor for root-forming substances other than auxin has opened possibilities for further experiments. For instance, it has been known for some time that cuttings of juvenile forms of many difficult plants root with ease. (See Gardner, 1929; Stoutemyer, 1937; Thimann and Delisle, 1939; Gardner and Piper, 1943). It seems possible that in certain cases grafts of juvenile shoots might be employed, coordinately with auxin to induce root formation in cuttings of old trees of species which are hard to root.

SUMMARY

Ruth Wilcox is a difficult-to-root white-flowered form of hibiscus. It does not respond to regular auxin treatments (fig. 1, table 1). However, if a shoot of an easy-rooting red hibiscus is grafted on cuttings of the white form an abundance of roots is formed provided the base of the white cuttings is treated with auxin (fig. 2, table 1). Without additional auxin treatment the grafted hibiscus shoot is incapable of producing roots.

These experiments show that for root formation

on the cuttings of the white form two factors are necessary: (1) auxin, and (2) a factor (or complex) present in the leaves of red hibiscus. The downward transport of the second substance takes place through the bark and is not strictly rectilinear (fig. 5 and 6, table 1). Only further research will tell whether the root-forming effect of red hibiscus leaves is hormonal or nutritional or both.

INSTITUTE OF TROPICAL AGRICULTURE,
MAYAGÜEZ, PUERTO RICO

LITERATURE CITED

- BEAL, J. M. 1940. Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. *Bot. Gaz.* 102:366-372.
- COOPER, W. C. 1935. Hormones in relation to root formation on stem cuttings. *Plant Physiol.* 10:789-794.
- . 1936. Transport of root-forming hormone in woody cuttings. *Plant Physiol.* 11:779-793.
- . 1938. Hormones and root formation. *Bot. Gaz.* 99:599-614.
- . 1944. The concentrated-solution-dip method of treating cuttings with growth substances. *Proc. Amer. Soc. Hort. Sci.* 44:533-541.
- , AND J. B. MANTON. 1937. Effect of hormones on the rooting of *Chrysanthemum* cuttings. *Florist's Rev.* July 29.
- GARDNER, F. E. 1929. The relationship between tree age and the rooting of cuttings. *Proc. Amer. Soc. Hort. Sci.* 26:101-104.
- , AND R. B. PIPER. 1943. Ease of propagation of some subtropical fruits by cuttings from young seedlings. *Proc. Florida State Hort. Soc.* (reprint without volume and page no.).
- GREGORY, L. E., AND J. VAN OVERBEEK. 1945. An analysis of the process of root formation on cuttings of a difficult-to-root *Hibiscus* variety. *Proc. Amer. Soc. Hort. Sci.* 46 (in press).
- HITCHCOCK, A. E., AND P. W. ZIMMERMAN. 1940. Effects obtained with mixtures of root-inducing and other substances. *Contrib. Boyce Thompson Inst.* 11:143-160.
- STOUTEMYER, V. T. 1937. Regeneration in various types of apple wood. *Agric. Exp. Sta. Iowa State Coll. Res. Bull.* 220:308-352.
- THIMANN, K. V., AND A. L. DELISLE. 1939. The vegetative propagation of difficult plants. *Jour. Arnold Arboret.* 20:116-136.
- , AND E. F. POUTASSE. 1941. Factors affecting root formation of *Phaseolus vulgaris*. *Plant Physiol.* 16:585-598.
- VAN OVERBEEK, G. 1944. Growth-regulating substances in plants. *Ann. Rev. Biochem.* 13:631-666.
- , M. A. TIÓ, L. E. GREGORY, AND E. M. SANTIAGO DE VÁZQUEZ. 1943-44. Plant physiological investigations. *Ann. Report of the director of the Inst. Trop. Agricult. Mayagüez, Puerto Rico, for 1943-1944.*
- WENT, F. W. 1929. On a substance causing root formation. *Proc. Kon. Akad. Wetensch.* 32:35-39.
- . 1933. Recherches expérimentales sur la néoformation des racines dans les plantules et les boutures des plantes supérieures. *Ann. Jard. Bot. Buitenzorg.* 43:87-168.
- . 1938. Specific factors other than auxin affecting growth and root formation. *Plant Physiol.* 13:55-80.
- . 1939. Growth hormones in the higher plants. *Ann. Rev. Biochem.* 8:521-540.
- , AND K. V. THIMANN. 1937. *Phytohormones.* Macmillan, N. Y.
- WILCOX, E. V., AND U. S. HOLT. 1913. Ornamental *Hibiscus* in Hawaii. *Hawaii Agric. Exp. Sta. Bull.* 29.
- YERKES, G. E. 1932. Propagation of trees and shrubs. *U. S. Dept. Agric. Farmer's Bull.* 1567.

NEW EVIDENCE ON THE TELOPHASE SPLIT IN *TODEA BARBARA*¹

Irene Manton

ULTRAVIOLET MICROSCOPY has been used sparingly in cytology in recent years (Lucas and Stark, 1931; Cole and Sutton, 1941). The photographs which accompany this paper illustrate the type of results which are being obtained with ultraviolet light, of $275\text{ }\mu$ wave length, by means of new methods recently devised for the study of spiral structure in the somatic chromosomes of *Osmunda* (Manton and Smiles, 1943). In extending the enquiry to the closely related genus *Todea*, no further change in technique has been introduced, and the reader is therefore referred to the paper on *Osmunda* for full details. Some knowledge of the general principles involved is necessary, however, for the critical assessment of the new data, and the procedure will be described in outline. The apparatus used is that at the National Institute of Medical Research, Hampstead, London, and I am deeply indebted to the authorities and staff of the Institute for research facilities freely placed at my disposal.

The genus *Todea* is very near to *Osmunda* taxonomically and resembles it so closely cytologically that for most purposes the two are indistinguishable. Unpublished photographic evidence shows that the chromosomes of *Todea* agree with those of *Osmunda* in number, size, distribution of chiasmata, and in details of spiral structure at most stages of mitosis and meiosis. The published photographs of *Osmunda* (Manton, 1939; Manton and Smiles, 1943) may, therefore, be quoted to illustrate the general manifestations of spiral structure in both genera; and the somewhat different response which one tissue of *Todea* has given to the spiral structure treatment, and which has provided the new evidence to be described, is not thought to indicate any fundamental difference between them.

MATERIAL AND METHODS. Treatment of the specimen.—The new evidence to be described here refers to a single preparation made from a culture of freshly gathered spores of the fern *Todea barbara*, sown in water at 80°F . in January, 1942. On the fifth day from sowing most of the spores had germinated and were in various stages of the third nuclear division. They were then fixed over-night in 1:3 acetic-alcohol after preliminary treatment for spiral structure. The preliminary treatment consisted in immersing the living spores in 50 per cent alcohol made alkaline with a drop of strong ammonia for six minutes before fixation.

After fixation the spores were smeared in acetocarmine (cf. Manton and Smiles, 1943) and the coverslip was ringed. The preliminary observations were carried out on the liquid mount and a number of photographs were taken. The specimen was then transferred to Canada balsam by McClintock's method.

Some of the photographs of one cell in late anaphase were published in a note in *Nature* (Manton, 1942). A year later the balsam was dissolved away and the same cell was examined photographically with ultraviolet light after removal to a quartz slide by means of Welch's stripping method (cf. Manton and Smiles, 1943). Before exposure to the ultraviolet light the cell was decolorized by soaking for some hours in 45 per cent acetic acid and was then mounted for photography in that liquid. Careful comparison of the two sets of photographs both of this and of other cells in the preparation show very close agreement between them except for a certain amount of shrinkage of the specimen. Such shrinkage was encountered on the previous occasion and is somewhat more marked here owing to the additional handling involved in the interim balsam mounting. In spite of this, the improved resolution in the ultraviolet photographs provides so much new information that only a selection will be published.

LATE ANAPHASE OF THE THIRD SPORE DIVISION.—Figures 1 and 2 are the two visual light photographs published in *Nature* (Manton, 1942). They represent corresponding parts (though not the whole nucleus in either case) of two superposed sister plates of chromosomes. The protoplasm in which they lie is still undivided but it has been squeezed out of its cell wall by momentary pressure applied to the coverslip for this purpose, in making the original acetocarmine mount. The chromosomes are not unduly damaged although some of them, especially those pointing approximately along the direction of movement of the protoplasm, have been considerably stretched—a stretching which has revealed significant structure. The chromosomes at right angles to the direction of flow have been far less distorted, and they still retain recognizable traces of spiral structure (as the ultraviolet photographs will best reveal), to the extent which is customary at this stage.

Figure 3 is an ultraviolet photograph at approximately the same focal level as figure 1 and at the same magnification. The object appears inverted, owing to the fact that it was turned over in remounting for the ultraviolet microscope in order to minimize the thickness of protoplasm overlying it. The shrinkage will also be noticed.

Figures 4a–6d contain selected details from ultraviolet photographs at twice the magnification of the preceding—a degree of enlargement which is legitimate with the improved resolution attained. Three separate sequences are represented out of the complete series of 32 significant exposures made at different focal levels through the whole extent of both the superposed nuclei. In each sequence the different levels are distinguished by letters of the alpha-

¹ Received for publication January 15, 1945.



Fig. 1-3. Chromosomes at anaphase of the third spore division in *Todea barbara*, $\times 2000$.—Fig. 1-2. The two photographs published in *Nature* (Manton, 1942) showing corresponding parts of sister plates of chromosomes.—Fig. 3. The same nucleus as in figure 1, photographed with the ultraviolet microscope. For further description see text.

bet, the distance between adjacent levels being 0.2μ in each case.

The sequence of figures 4 a-d may be passed over briefly since it contains the chromosomes previously commented upon in *Nature*. The peripheral chromosomes, pointing in the direction of movement of the protoplasm, have been very much stretched and in one of them "two distinct and separate threads have been forced widely asunder" (Manton, 1942). These two threads are still visible in the ultraviolet series though they now lie less conveniently in one focal plane. Stress will not, however, at the moment be laid upon them, for their validity as evidence of splitting has been disputed (Koller, 1942; Thomas, 1942) on the ground that the particular chromosome concerned was not a normal one but was a dicentric bridge. My opinion that such an interpretation is unfounded has already been expressed (Manton, 1942a) and it is not necessary to deal with this dispute in detail here for at most it affects only the one chromosome.

The sequence of figure 5 a-e is from the sister nucleus of figure 2 though at a lower focus than that figure. The most instructive chromosome is that marked by an arrow in figure 5 a and c; it lies roughly at right angles to the direction of figure 4 and it is relatively undistorted. Two observations of importance can be made on this chromosome and to some extent on others in the field. In the first place the light central region, visible in figures 5 b-d, is undoubtedly a space since it contains no light-absorbing material at any focal level. Secondly, the oblique lines seen on the chromatic structures on either side of this space (see especially the right side of the chromosome in figure 5d) are undoubtedly the gyres of spiral structure. This is indicated first by comparing them with the somatic chromosomes of *Osmunda* in which the number and approximate size of the gyres have been ascertained with certainty; and second by the fact that direction of coiling can be determined, in part of the chromosome, by observing the changes of apparent slope of the gyres at different focal levels. The slope of the arrows in figures 5 a and c is similar to that of the gyres to which they point and for at least three turns in the left half-chromosome the direction of coiling is right handed.

The importance of figure 5 a-e is first in the confirmation it provides that two distinct and sepa-

table, if not separated, threads are present in one of the least distorted of the chromosomes. To what extent the actual degree of separation observed has been enhanced by the treatment is uncertain but whether natural or artificial it is of considerable interest to find that the two half-chromosomes can separate laterally while still in the spiral configuration without interlocking at the gyres of the spiral. The probable meaning of this is discussed below.

Figure 6 a-d is from another part of the same nucleus but from the opposite side and therefore also unaffected by the dispute. The chromosomes in this sequence are again stretched, though to a less extreme degree than those of figure 4. The spiral structure has been effaced, or nearly so, by the stretching, as in parts of figure 4, though in a few places it is detectable, e.g., near the tip of the central chromosome where the coiling in each half is almost certainly left-handed. The central cleft is again visible, in this case most clearly seen near the end of the middle chromosome of figure 6c; but in other places the number of distinct and separate threads can be seen to be, not two, but no less than four. All four are simultaneously detectable at the distal end of the upper chromosome in figure 6c while three strands can be seen simultaneously in several places in this and other focal levels.

If it were not for the dispute concerning the nature of the split chromosome of figure 4, the most natural interpretation of the various appearances just enumerated would be that they all represent chromosomes of fundamentally similar structure showing progressively increasing effects of stretching in the order figure 5, figure 6, figure 4; the disputed chromosome of figure 4 being merely the most extreme case of deformation produced on the fixed material by the method of mounting. This interpretation is, in my opinion, still the most probable one. Even without figure 4, however, the evidence for many-strandedness is plain enough. It is impossible to know whether further increase of resolution would detect additional subdivisions in the quarter-chromosomes of figure 6. It is, however, clear from this cell that the chromosomes of *Todea* are not single, coiled threads, but that they are at least quadripartite at a late stage of anaphase.

LATE PROPHASE OF THE THIRD SPORE DIVISION.—

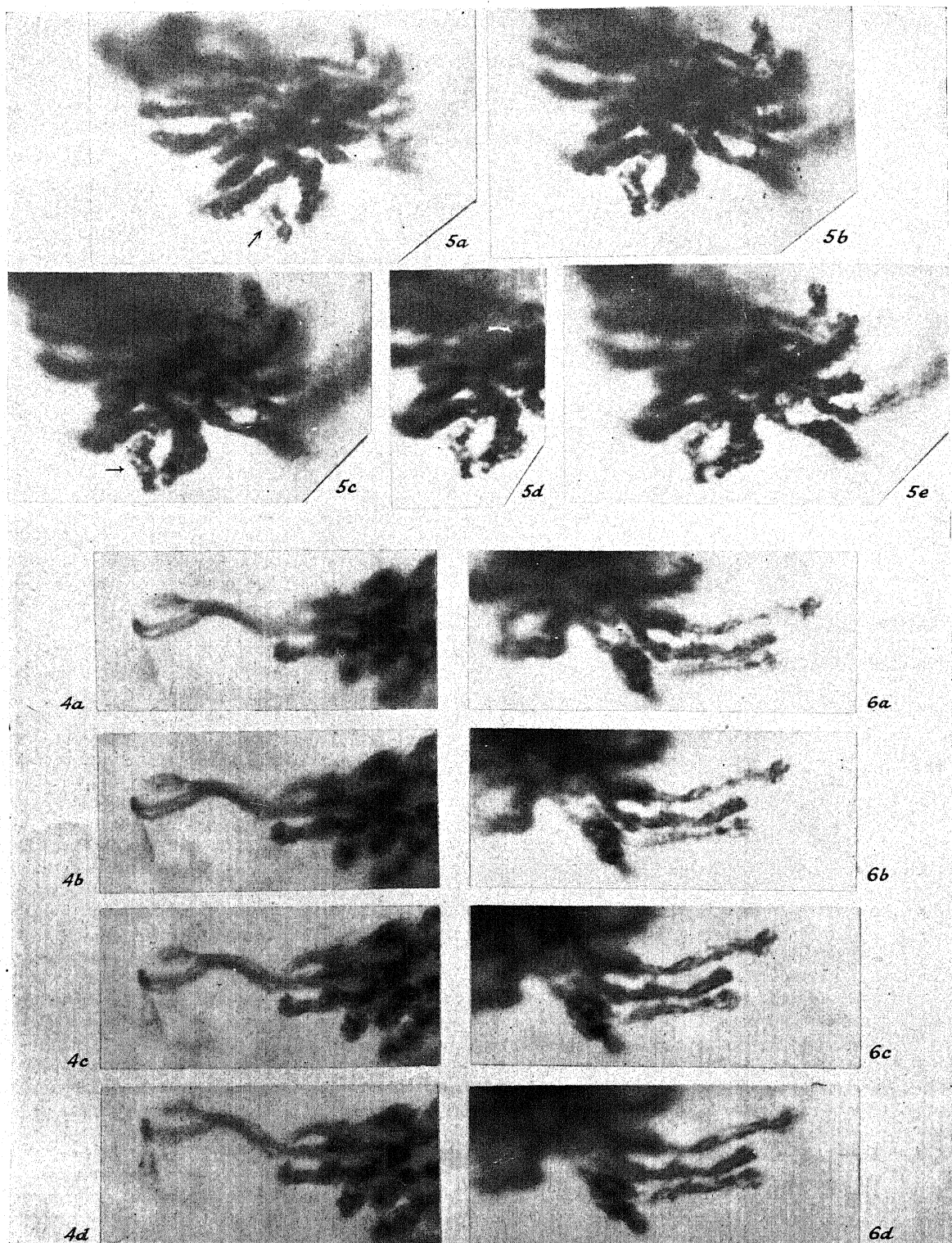


Fig. 4-6. Ultraviolet photographs of the same cell seen in figures 1-3, $\times 4000$.—Fig. 4 a-d. Sequence of exposures 0.2μ apart through the disputed region on the left of figures 1 and 3.—Fig. 5 a-e. Sequence of exposures 0.2μ apart through a part of the sister nucleus to figure 4.—Fig. 6 a-d. Sequence of exposures 0.2μ apart from the nucleus shown in figure 5. Further description in text.

Figures 7–15 are from a prophase nucleus originally on a different part of the same preparation as the preceding and therefore belonging to a different prothallus. As before, the protoplasm and nucleus had been squeezed out of their cell wall in making the mount but in this case the cell contents had become separated from the rest of the spore. There is, therefore, only presumptive evidence, from the general uniformity of the culture, as to which division is concerned, but it was probably the third.

The nucleus is very beautifully displayed, the pole field being uppermost, and there is no detectable deformation in it. Figure 7 shows it in the stained condition in balsam, figure 8 gives a larger view of the upper surface as seen with visual light in the original acetocarmine mount, while figure 9 is a corresponding level as seen with the ultraviolet microscope. The shrinkage recorded in the preceding specimen is again displayed.

One chromosome in the visual light photograph (fig. 8) is marked with arrows. The centromere of this chromosome is practically terminal and the two chromatids lie well apart in the upper half of the chromosome, though they twist about each other below. Their knobby appearance is certainly related in some way to the developing spiral structure, though the exact details of this relationship are not fully elucidated at this stage. Between the arrows, however, not two but four separate strands were detectable even with visual light. Three are in focus in the plane of figure 8 and a fourth lay at a deeper level. This chromosome unfortunately proved to be somewhat opaque to ultraviolet light and the three focal levels reproduced as figure 10 a–c are less fully informative than are some of the other chromosomes elsewhere in the plate; they may nevertheless have some comparative interest.

The most instructive region of the nucleus when studied with ultraviolet light is that near the central arrow in figure 11. The focal level here is low and close to the bottom surface of the nucleus. The distal ends of several chromosomes are seen, in part overlaid by the shadow of the nucleolus which lies above. Enlarged details appear in figures 13, 14, and 12 a–f.

In all the chromosomes distinguished by arrows in figures 12–14, and perhaps elsewhere also, a chromatid is resolved into two separate components, in the original prints at any rate. The most extensive length of separation is in the lower chromatid of the right hand chromosome of figure 12 c–d. This separation was also just detectable in the corresponding visual light photograph though it is doubtful whether the appearance would survive the process of half-tone reproduction and it is not included in the plate. Traces of spiral structure are also manifested in this chromatid.

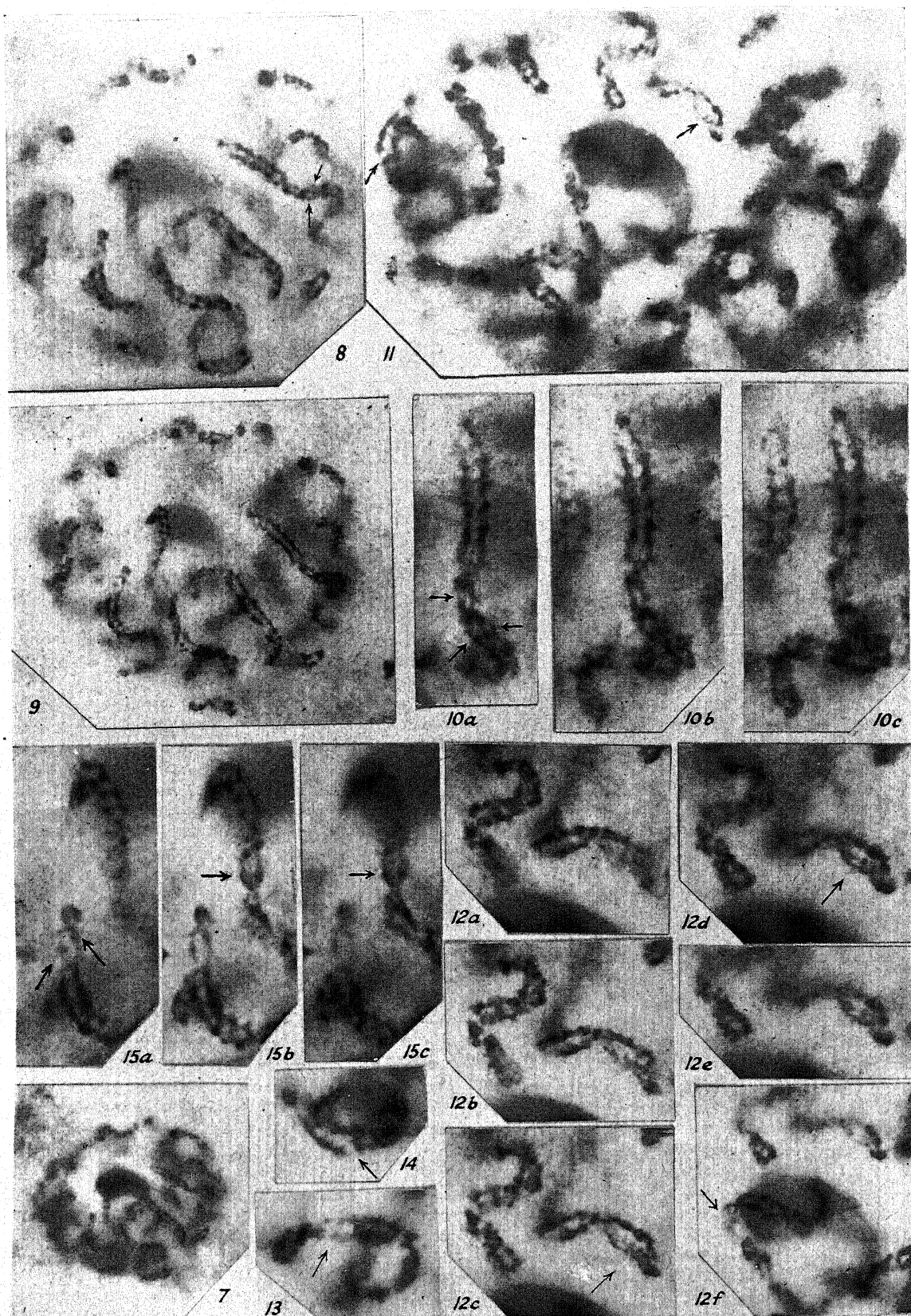
In all these examples the components of the chromatids are approximately equal in thickness; in certain places, however, they are unequal. Thus in figure 15 b–c a delicate thread has separated from a stouter one in the part of the upper chromosome

marked with an arrow. Whether such a delicate strand is less than a half-chromatid or owes its thinness to some other cause is not known. It is obvious that at this point the detection of quarter-chromatids, if they exist, must again be curtailed by the limits of resolution even of light of short wave length. It is, however, unnecessary to push the indications of finer subdivision any further, for even half-chromatids are sufficient for the present purpose. The evidence from the whole of this nucleus indicates that in prophase no less than in anaphase a chromosome of *Todea* seems to be at least quadripartite.

Before leaving this plate, attention may be called to another detail in figures 15 a–c. These figures show the centromere ends of two chromosomes which are in their setting at the left of figures 8 and 9. The region of the centromere is in focus in the lower of the two chromosomes in figure 15 a and b. The chromatids here, as in the upper end of the chromosome of figure 10 a–c, are very thin and thread-like, but in figure 15b two granular objects at slightly different levels are borne on the two sides. That these objects are probably not granules but gyres of a tiny spiral is suggested by the fact that both resolve into two diagonal lines at a lower focal level. These diagonal lines are approximately in focus in figure 15a though the left side is more so than the right. The slope of the diagonal lines is indicated by the arrows.

INTERPRETATION OF THE EVIDENCE.—While allowance must be made for some loss of clarity in reproduction it is hoped that the many-strandedness at both prophase and anaphase has been demonstrated. The question may be legitimately raised, however, as to the extent to which the appearances are artifacts and therefore misleading. That artificial appearances are not necessarily misleading is of course familiar to every student of spiral structure, for the clarity with which the gyres are revealed is almost always artificial though the spiral itself is not.

It is perhaps legitimate to suggest that the observations just described are trustworthy as evidence for the points raised in the same sense as were the observations on spiral structure made after exactly similar treatment in *Osmunda*, observations whose trustworthiness has not been questioned. In any case it is certain that the details recorded above cannot have been introduced into the specimens by any of the processes used after the original mounting in acetocarmine. It is also certain that the acetocarmine mounting alone does not usually cause comparable manifestations of structure, in this or related material, without pre-treatment with ammoniated alcohol. Considerable experience of the particular pre-treatment in use, on germinating fern spores, has shown that obviously pathological effects are not involved unless longer periods of treatment or higher temperatures are employed, and then such effects are of a very different kind and easily recognizable. Thus chromosomes may become clumped



together, swollen, attenuated, pulled out into apparently structureless threads or occasionally transversely broken by more extreme treatments or more violent handling, but nothing in the specimens described above suggests that either abnormality or damage on the scale required has affected them. That similar appearances were not revealed in *Osmunda* by similar treatment (cf. Manton and Smiles, 1943) is most easily explained, not by postulating normality in the one case and gross artifacts in the other, but by the probable existence of a relatively minor difference in the powers of cohesion of the components of the chromosomes in the two plants. The gyres separate under the ammonia treatment in both cases, but in *Todea*, unlike *Osmunda*, the lateral cohesion of longitudinally running component strands is also affected. The extent to which such component strands were spatially separated in life is unknown. There is, however, nothing to warrant the assumption that they could have been produced by the treatment.

THE THEORETICAL CONSEQUENCES OF THE EVIDENCE, IF CORRECT.—If the interpretation of the evidence here adopted is correct the results have a close bearing on current problems of spiral structure. It has been thought by many workers, myself included (cf. Manton, 1939), that the spiral involved in a somatic division must differ in some fundamental respects from that at meiosis, for whereas the component chromatids involved in the large-coiled "major" spiral at the first meiotic division can separate laterally without difficulty, the apparent spatial relations of the two chromatids at an early somatic prophase seem to indicate that they are twisted together and closely interlocked. The difficulties entailed by such an interpretation have, however, become increasingly apparent in recent years; and in a case such as the ring chromosomes of McClintock (1941), in which lateral separation of half chromosomes occurs readily, though their geometrical form would appear to preclude the possibility of unwinding, the idea of interlocking spirals would appear to be untenable.

The terminology invented to describe the two forms of spirals is somewhat confused owing to simultaneous publication by different authors in different countries. My own terminology (Manton, 1939) was "twisted spiral" and "non-torsional spiral" for the types thought to characterize mitosis and meiosis respectively; Kuwada's (1940) terms for these are "orthospiral" and "anorthospiral"; Matsuura (1941) uses "relational-double-thread-spiral" and "parallel-double-thread-spiral,"

while Sparrow, Huskins, and Wilson (1940) have put the same idea into Greek in the adjectives "plectonemic" and "paranemic." Since the terminology of Sparrow, Huskins, and Wilson appears to be the least liable to misunderstanding I propose to adopt it.

There is little reason to doubt that the spiral at the first meiotic division in most plants (cf. Huskins, 1941, etc.), and certainly in the Osmundaceae (see Manton, 1939) is paranemic. The evidence from the somatic chromosomes of *Todea* now indicates that, contrary to expectation, the somatic spiral is also paranemic. This evidence is not only strong in itself but is in full accord with much of the work summarized by Nebel (1941), as well as with the behavior of McClintock's ring chromosomes already mentioned. This weight of evidence appears to be very nearly, if not quite, conclusive.

Abandonment of the idea of the plectonemic spiral will obviously entail the finding of some other interpretation for the early prophase appearances on which the idea was originally based. This should not prove impossible, for the critical observational details involved lie nearer to the limits of visibility than does the other evidence now available, and therefore the chance of misinterpretation having previously occurred is proportionately greater.

The acceptance of many-strandedness would also be of importance in another connection. The argument for or against the "telophase split" would then seem to be unreal. If a chromosome is at all times multiple, "splitting" in any literal sense may never occur at all. The multiplication of unit strands, if viewed as a chemical problem, may, but need not, involve fission of the smallest submicroscopic threads. Chromosome bipartition, on the other hand, may possibly only entail the spatial separation of bundles of strands each of which has long had an individual existence. Expressed another way, the central problem of mitosis may be reformulated in two precise questions: (1) By what means are a number of potentially separate threads coordinated into taking a common spiral path? (2) Why are there two such spiral paths at prophase, developed so independently of each other that they may even (Manton and Smiles, 1943) be coiled in opposite directions? No answer can be made to these questions as yet, but in formulating them one is perhaps drawing nearer to an understanding of the processes involved in nuclear division than would be possible by merely discussing the reality of the telophase split in the old terms.

Fig. 7-15. Late prophase of the third spore division in *Todea barbara*.—Fig. 7. The nucleus stained and in balsam, visual light, $\times 1000$.—Fig. 8. Upper surface of the nucleus in acetocarmine, visual light, $\times 2000$.—Fig. 9. The same as figure 8, photographed with ultraviolet light, $\times 2000$.—Fig. 10 a-c. Ultraviolet photographs of the marked chromosome in figure 8. $\times 4000$. Focal levels approximately 0.3μ apart.—Fig. 11. Ultraviolet photograph near bottom surface of nucleus, $\times 3000$.—Fig. 12-14. Details of marked regions in figure 11.—Fig. 12 a-f. Sequence of exposures 0.2μ apart of region near central arrow in figure 11, $\times 4000$, except for figure 12f, which is $\times 3000$.—Fig. 13. Distal end of the chromosome visible in bottom right hand corner of figure 12f, ultraviolet photograph, $\times 4000$.—Fig. 14. Detail of chromosome marked by left hand arrow in figure 11, $\times 4000$.—Fig. 15 a-c. Detail of centromere ends of two chromosomes visible on left in figures 8 and 9; ultraviolet photographs, approximately 0.5μ apart, $\times 4000$.

Further evidence on these topics will be published elsewhere.

SUMMARY

In young prothallial material of the fern *Todea barbara*, treated for spiral structure, a cell previously photographed and described in a preliminary form in a note to Nature (Manton, 1942) has been re-examined with the ultraviolet microscope. Much additional information on chromosome structure is revealed which is thought to be significant.

In the previously described cell, which is a late anaphase of the third mitosis in the germinating spore, not less than four component strands appear

to be present in some chromosomes, while in others half-chromosomes which are still in the spiral configuration appear able to separate laterally without interlocking. The probable meaning of both of these observations is discussed.

This evidence has been amplified by observations with both visual and ultraviolet light on a late prophase nucleus from another prothallus in the same material. Evidence of many-strandedness is again found and its relation to the problem of the "telophase split" is discussed.

BOTANY DEPARTMENT,
THE UNIVERSITY,
MANCHESTER, ENGLAND

LITERATURE CITED

- COLE, P. A., AND E. SUTTON. 1941. The absorption of ultraviolet radiation by bands of the salivary gland chromosomes of *Drosophila melanogaster*. Cold Spring Harbor Symposia on Quantitative Biology 9:66-71.
- HUSKINS, C. L. 1941. The coiling of chromonemata. *Ibid.*:13-17.
- KOLLER, P. C. 1942. The telophase split in *Todea*. *Nature* 150:736.
- KUWADA, Y. 1940. Chromosome structure. A critical review. *Cytologia* 10, p. 213-256.
- LUCAS, F. F., AND M. B. STARK. 1931. A study of living sperm cells of certain grasshoppers by means of the ultraviolet microscope. *Jour. Morph.* 52:91-113.
- MANTON, I. 1939. Evidence on spiral structure and chromosome pairing in *Osmunda regalis* L. *Phil. Trans. Roy. Soc. London B.* 230:179-215.
- . 1942. Demonstration of the telophase split in *Todea*. *Nature* 150:547.
- . 1942a. The telophase split in *Todea*. *Nature* 150:736.
- , AND J. SMILES. 1943. Observations on the spiral structure of somatic chromosomes in *Osmunda* with the aid of ultraviolet light. *Annals Bot. N.S.* 7:195.
- MATSUURA, H. 1941. Chromosome studies in *Trillium Kamtschaticum* Pall. XV. A contribution to the present state of knowledge on the mechanism of chromonema coiling. *Cytologia* 11:407-428.
- McCLINTOCK B. 1941. Spontaneous alterations in chromosome size and form in *Zea mays*. Cold Spring Harbor Symposia on Quantitative Biology 9:72-81.
- NEBEL, B. R. 1941. Structure of *Tradescantia* and *Trillium* chromosomes with particular emphasis on number of chromonemata. *Ibid.*:7-12.
- SPARROW, A. H., C. L. HUSKINS, AND G. B. WILSON. 1941. Studies on the chromosome spiralization cycle in *Trillium*. *Canadian Jour. Res.* 19:323-350.
- THOMAS, P. T. 1942. The telophase split in *Todea*. *Nature* 150:736.

ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

Reprinted by permission of the authors from: RIKER, A. J., and REGINA S. RIKER. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.

ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

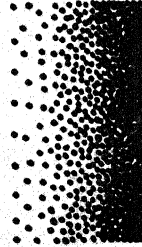
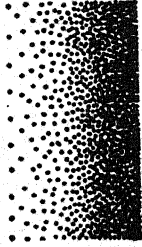
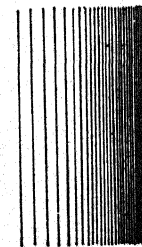
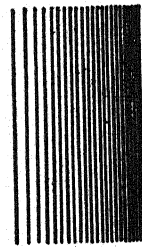
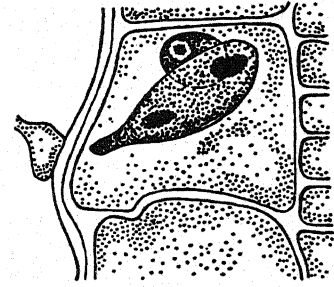
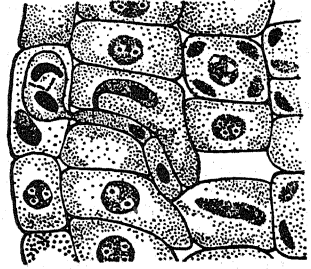
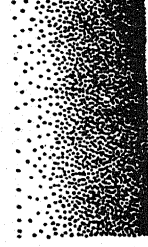
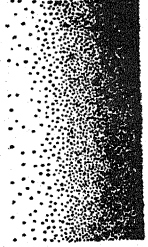
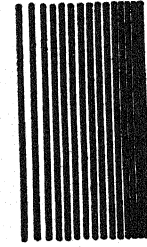
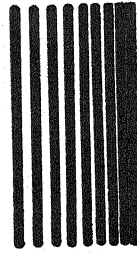
ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



THE USE OF THE C^{13} ISOTOPE AS A TRACER FOR TRANSPORT STUDIES IN PLANTS ¹

G. S. Rabideau² and G. O. Burr

CURRENT VIEWS on the rate, mechanism and site of translocation of organic and inorganic materials in plants are based on the results of experiments of many types. The various tissues and organs of normal and surgically or chemically treated plants have been quantitatively analyzed either for normal constituents (e.g., sugars, organic nitrogen, hormones, vitamins or salts) or for introduced indicators such as dyes, viruses and isotope-containing compounds. The results of this work have been summarized and critically discussed in several recent reviews (Münch, 1930; Curtis, 1935; Mason and Phyllis, 1937; Both, 1937; Dixon, 1938; Crafts, 1938, 1939).

Much of the recent work on translocation and uptake of inorganic materials by plants has been done with isotopic tracers. The first study of plant transport using radioactive materials was made by Hevesy (1923) with radioactive lead. Gustafson and Darken (1937) and Gustafson (1939) found evidence of upward movement of minerals in both the phloem and xylem of *Sedum* and *Bryophyllum*, using radioactive phosphorus, P^{32} . Stout and Hoagland (1939) studied ion uptake and demonstrated lateral transport of radioactive phosphorus, sodium, and potassium in barley plants. Biddulph (1941) showed that the upward movement of radioactive phosphorus takes place in the xylem tissue of bean plants. Biddulph and Markle (1944) found that the downward movement of radioactive phosphorus from cotton leaves occurs in the phloem tissue. Colwell (1942) reported certain conditions under which the transport of P^{32} absorbed by the leaves of squash plants could be limited to the phloem of killed stems. Overstreet and Broyer (1940) have used radioactive carbon, C^{11} , in bicarbonate in studying ion exchange by the roots of barley plants.

Because of their obvious advantages, the mass isotopes of carbon, hydrogen, oxygen and nitrogen and the radioactive isotope of carbon, C^{11} , have been used in many studies of intermediary metabolism, too numerous to mention here. C^{11} serves well as a tracer in short-time studies (Van Niel *et al.*, 1942), but its half-life of 21 minutes limits its use.

¹ Received for publication February 26, 1945.

The authors gratefully acknowledge the assistance of Dr. A. O. Nier of the physics department and Dr. Wallace Armstrong of the department of physiological chemistry. Grants from the Rockefeller Foundation and the Graduate School of the University of Minnesota made this work possible.

² The experimental data in this paper are taken from a thesis submitted to the faculty of the Graduate School of the University of Minnesota by G. S. Rabideau in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

fulness for measuring translocation. Heavy carbon, C^{13} , can be used over any period of time and the samples stored for analysis.

Belkengren (1941) used the C^{13} isotope as a tracer in photosynthesis and translocation studies with young bean plants. He measured the rate of movement of newly-assimilated C^{13} from leaf to root, and the rate of accumulation of C^{13} in several chemical fractions of the root, stem, and leaf. Both observations yielded evidence that about 2 hours are required for the synthesis of carbohydrate and its transport in sufficient quantity to be detected at a distance of 30 cm. from the point of synthesis. Exposure of the leaves to tagged CO_2 in the dark failed to demonstrate transport of unassimilated CO_2 in the inorganic fraction, thus confirming the work of Miller and Burr (1935).

In the present paper, CO_2 enriched with C^{13} is used as a tracer in order to study (1) the limitation of the movement of photosynthate to living tissue, and (2) the degree of cross transport of the labeled photosynthate between the primary leaves of the bean plant.

EXPERIMENTAL METHODS.—The bean, *Phaseolus vulgaris* L. hort. var. Kentucky Wonder, was grown by the liquid culture method using a Shive four-salt solution as modified by Wolkoff (1918):

1.5 millimoles $(NH_4)_2SO_4$ per liter of culture solution
2.7 millimoles KH_2PO_4 per liter of culture solution
7.5 millimoles $MgSO_4 \cdot 7H_2O$ per liter of culture solution
10.0 millimoles $Ca(NO_3)_2 \cdot 4H_2O$ per liter of culture solution.

To each liter of the above culture solution there was added a 5 ml. portion of the following trace element solution:

0.08 millimoles $CuSO_4 \cdot 5H_2O$
0.6 millimoles $MnSO_4 \cdot 7H_2O$
0.6 millimoles $ZnSO_4 \cdot 7H_2O$
2.6 millimoles H_3BO_3
1 liter of distilled water.

A few milligrams of ferric citrate were added to each culture jar as a source of iron. The bean seeds were soaked, husked, and placed in petri dishes over moist filter paper until the roots were about 2 cm. long. They were then placed on perforated iron plates or large mesh screen and set over jars containing the above culture solution. Any support needed by the stems was furnished by rings of plasticine which did not injure the tissues. These plants were grown at room temperature under continuous illumination from a bank of tungsten bulbs. A 19 cm. water filter as used by Beber (1937) removed most of the harmful infra-red light. The incident intensity on the leaves was about 100 foot-candles

when measured with a photronic cell. The plants were ready for use when about 15 days old. At this time each leaf was about 50–75 sq. cm. in area; the stem (root to primary leaves) was about 20 cm. long; and the stem tip (primary leaves to stem apex) was about 6–10 cm. long, with the secondary leaf at, or very near, the apex of the stem tip. During some of the experiments the primary leaves were enclosed in separate glass photosynthesis chambers, each of 1.2 liters volume, using soft

from this BaCO_3 as needed by the addition of dilute lactic acid, and forced into the photosynthesis chambers from a burette by displacement with mercury.

A Carpenter-Haldane (1923) gas analysis apparatus with a burette volume of 40 cc. was used to determine the initial and final concentrations of CO_2 in the photosynthesis chambers. In addition, whenever possible, a sample of the chamber gas was drawn through NaOH by aspiration and the absorbed CO_2 was analyzed with the mass spectrometer to give the initial concentration of C^{13} . In some cases, enough CO_2 remained in the photosynthesis chamber at the close of the experiment to afford a final analysis for C^{13} .

A modified Van Slyke apparatus (fig. 1) and the reagents of Van Slyke and Folch (1940) were used for the conversion of tissue carbon to CO_2 prior to isotopic analysis. The CO_2 sample collected in the burette was then allowed to flow into an evacuated bulb attached to the apparatus through a ground glass joint. These CO_2 samples were analyzed with a 60° mass spectrometer designed by Nier (1940) for rapid and accurate analysis of isotopes. The results of deflection measurements of mass peaks 45 and 44 were not expressed as per cent C^{13}O_2 in the total CO_2 , which would be:

$$\frac{\text{mass 45}}{\text{mass 44} + \text{mass 45}} \times 100,$$

but rather the ratio of:

$$\frac{\text{mass 45}}{\text{mass 44}} \times 100.$$

Since the mass spectrometer gives values of the latter ratio, and since the correction to per cent values is small for the relatively low ratios used in this work, these ratios were not corrected to per cent C^{13} . Nier and Gulbransen (1939) state that the relative abundance of C^{13} in ordinary atmospheric CO_2 is about 1.09 per cent, but that the heavy oxygen, O^{17} , present in the CO_2 increases this value to about 1.18 for the ratio of mass of 45 to 44×100 . The ratios reported in this paper were not corrected for oxygen, mass 17. The maximum error is ± 0.01 .

Since the mass spectrometer had a small amount of dark current which varied from day to day, values of a certain sample measured one day differed slightly from those obtained on the following day. To correct for this daily variation, it was customary to run a sample of standard CO_2 daily. This standard was prepared from a carbonate reserved for this purpose. It was assumed that there was no C^{13} variation in this standard sample and that any observed variation in its analysis was attributable to the mass spectrometer itself. By subtracting this small fluctuation from each sample value obtained on that day, ratios were obtained which could be compared directly with ratios obtained on other days.

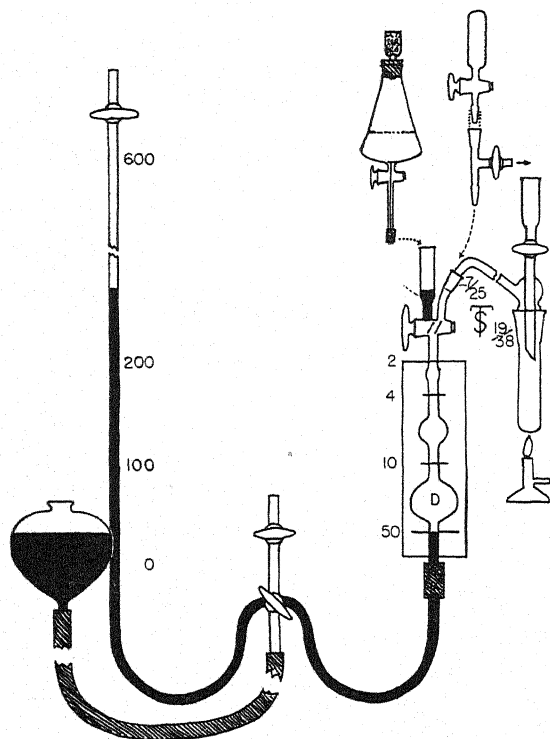


Fig. 1. The Van Slyke apparatus used for the combustion of leaf tissue samples. Modifications consist of a "tee" tube and a gas sample bulb attached at the 7/25 standard taper joint. This allowed for the evacuation of the sample bulb and the transfer of the carbon dioxide gas to the sample bulb.

plasticine to seal the petioles in the notched chambers. In this manner one or both leaves could be exposed to light and to CO_2 enriched with the C^{13} isotope. The movement of the tagged photosynthate to various plant parts was followed by acid digestion of selected tissues or tissue extracts, and the subsequent analysis of the CO_2 for heavy carbon with the mass spectrometer.

The CO_2 enriched with C^{13} was obtained from BaCO_3 prepared by the combustion of a heavy methane produced in the thermal diffusion column described by Nier and Bardeen (1941). The enriched CO_2 from the combustion of this methane was absorbed in NaOH and later precipitated as BaCO_3 by the addition of BaCl_2 solution. The carbonates prepared by this method contained 8 to 14 per cent of the C^{13} isotope. The CO_2 was liberated

RESULTS.—*Transport experiments with normal stem plants.*—Three healthy bean plants were selected. The paired primary leaves of plant 1 (fig. 2) were sealed in photosynthesis chambers and one leaf was exposed to enriched CO_2 . The fed leaf was

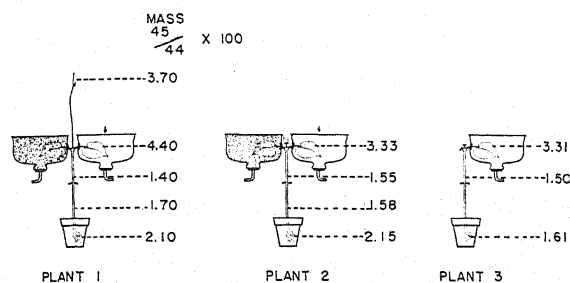


Fig. 2. Schematic representation of the results of transport experiments using C^{13} and normal plant stems. Plant 1 is entire, plant 2 has the stem tip removed, and plant 3 has both the stem tip and one leaf removed. Non-illuminated chambers are represented by stippled areas. Each figure is the mean of at least two determinations and is relative to a ratio of 1.16 ± 0.01 for normal plant tissue. The ratio of the CO_2 fed to the illuminated leaves is 9.0.

exposed to water-filtered tungsten light of about 300 foot-candles incident intensity while the unfed leaf was kept in the dark. After 43 hours exposure to light, the plant was removed and short sections of the stem, stem tip, root tips, and of each primary leaf were separately burned to CO_2 in the Van Slyke apparatus and the isotopic carbon ratio determined as described above.

The stem tip of plant 2 (fig. 2) was cut off just above the primary leaf node and the plant was subjected to the same conditions as plant 1, except that the duration of the experiment was 24 hours.

One primary leaf and the stem tip of plant 3 (fig. 2) were removed close to the primary leaf node, and the remaining primary leaf was enclosed in a glass chamber and exposed to labeled CO_2 and to light for a period of 18 hours.

The results, shown graphically in figure 2, indicate the amounts of new photosynthate present in each region. Removal of the tip or the opposite primary leaf did not stop the downward transport to the root tips.

Assuming that all new carbon compounds made from the fed CO_2 had a mass ratio of 9.0 it can be calculated that in plant no. 1 the analyzed tissues contained the following percentages of new carbon: stem tip, 32.4 per cent; leaf, 41.3 per cent; stem between leaf and cotyledon, 3.1 per cent; stem between cotyledon and root, 6.9 per cent; root tips, 12.0 per cent. Large amounts of labeled photosynthate were transported upward and downward to metabolically active regions. The little present in the stem may be largely soluble compounds in transit. The following attempt was made to fractionate the stem fluids. The stem above the primary leaves of a bean plant which had just concluded a period

of photosynthesis in enriched CO_2 was cut into three sections which were placed basal end downward in a medicine dropper. This dropper was mounted in rubber in a 10 cc. centrifuge tube and the whole assembly centrifuged at about 1500 r.p.m. for an hour to extract the "tracheal fluid" according to the method of Hamm *et al.* (1941). The fluid was dried and the residue, as well as samples of the stem tip taken before and after centrifugation, were oxidized to CO_2 in the Van Slyke apparatus and the isotopic ratio determined with the mass spectrometer. The results in table 1 show that this method does not effectively separate the stem fluids into fractions which are rich and poor in new photosynthate.

TABLE 1. Results of the "tracheal fluid" experiment. Each ratio of mass $45/44 \times 100$ is the mean of two determinations and is relative to an isotopic ratio of 1.16 for normal plant tissue.

Material	Mass $45/44 \times 100$
Fed stem before centrifugation	1.49
Fed stem after centrifugation	1.41
Fed stem centrifugate	1.43

Further investigation of this problem was made by the use of a method which shows the relationship between the amount of C^{13} in a tissue and its growth. The stem above the primary leaves of a normal bean plant was marked off in approximate

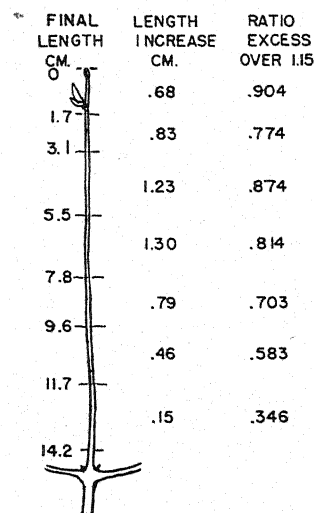


Fig. 3. Results of the stem elongation experiment. Column 1, final stem length. Column 2, increase in length over original stem length. Column 3, ratio increase over the ratio of 1.16 for CO_2 mass $45/44 \times 100$ for normal plant tissue.

millimeter divisions with India ink. The distances between the marks were measured and recorded. The primary leaves were then placed in photosynthesis chambers and exposed to incandescent lamp illumination and to heavy CO_2 for a period of 22

hours. The plant was then left in the dark for an additional 24 hours. The stem was 9 cm. in length when marked and 14.2 cm. in length after 46 hours, the duration of the experiment. The distances between the marks were remeasured and recorded. The stem was sectioned at the marks and each section was oxidized separately in the Van Slyke apparatus and its CO_2 analyzed for C^{13} . The results of this experiment are shown in figures 3 and 4.

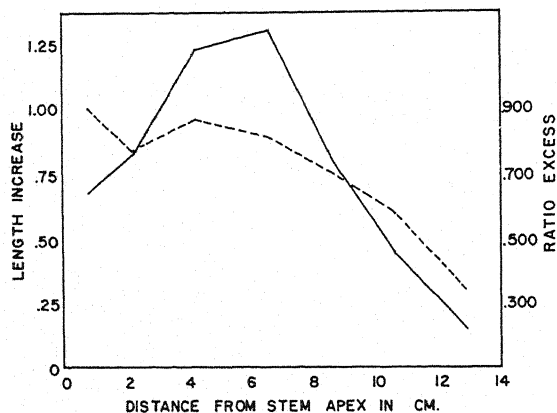


Fig. 4. Stem elongation experiment. Ordinate: solid line is the increase in stem length over the original length; broken line is the ratio excess of CO_2 mass $^{45}_{44}\text{C} \times 100$ over a normal tissue ratio of 1.16. Abscissa is the distance from the tip of the stem.

These results show a correlation between the ratio excess of C^{13} and the growth rate. The large accumulation of C^{13} in the meristematic region of the stem tip shows incorporation of the heavy carbon into the newly formed cells as well as into elongating cells. The two regions of greatest incorporation of new photosynthate (the extreme tip and the rapidly elongating region) are separated by a region of somewhat lower activity.

Transport experiments with plants having killed stems.—Three similar bean plants were chosen and sections of the stem were killed with hot wax according to the following method of Overton (1911): A pyrex glass tube of 2 cm. bore was slipped over the stem, the lower end plugged with a split cork and cotton, a thin layer of melted beeswax ($50^\circ\text{C}.$) was poured in and allowed to harden. This was followed by a layer of melted beeswax ($100^\circ\text{C}.$), which killed the adjacent tissue as does the scalding method described by Overton and used recently by Colwell (1942). Such plants lived and were able to carry on photosynthesis for several days after being treated in this manner.

Two 6 cm. sections of plant 4 were killed, in the middle of the stem above the primary leaf node, and in the middle of the stem below the primary leaf node (fig. 5). One 3 cm. section of the stem was killed on each of plants 5 and 6, the killed section being below the primary leaf node on plant

5 and above the primary leaf node on plant 6. At least one day was allowed to elapse between the killing and the exposure to tagged CO_2 . The primary leaves were then sealed in the photosynthesis chambers, and one leaf of each plant was exposed to light and to enriched CO_2 for 24 hours.

The results of burning sections of whole tissue in the regions indicated in figure 5 show that, although the leaves of the plants with killed stems

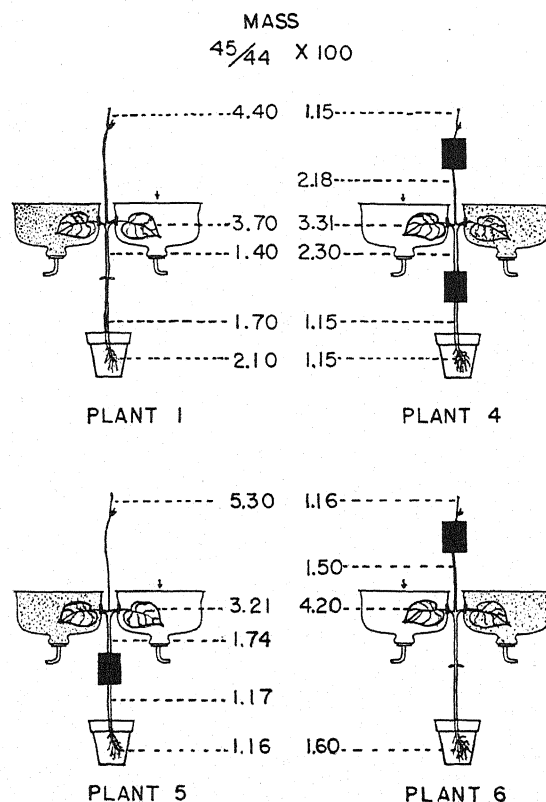


Fig. 5. Schematic representation of the transport of C^{13} through killed stems. Plant 1, a normal plant, is included for comparison. Unilluminated chambers are stippled, while killed stem sections are black. Each figure is the mean of at least two determinations and is relative to a ratio of 1.16 for normal plant tissue. Initial ratio of CO_2 introduced into chambers: Plant 1, 9.0, plant 2, 10.2, plants 3 and 4, 9.1.

carried on photosynthesis and accumulated high concentrations of C^{13} , no detectable amount of isotopic photosynthate was conducted through the killed areas in contrast to the ready conduction by normal tissues. Subsequent combustion of the killed section itself showed no excess of C^{13} in this region, indicating the effectiveness of the stoppage of movement of photosynthate. An extension of this experiment using a more sensitive method for the detection of the labelled photosynthate consisted of burning the solids obtained by 80 per cent alcohol extraction of the stem section above and below the killed areas. These results are given

in table 2. They further demonstrate that photo-

TABLE 2. Ratio analyses of soluble carbohydrate extracts (80 per cent alcohol) of the stem sections. Experiment A, wax-killed stem whose leaves were exposed to C^{13} , and experiment B, a normal stem whose leaves were not exposed to C^{13} .

Experiment	Location of sample	Mass $45/44 \times 100$
A	Killed section to fed leaf....	2.50
	Below killed section.....	1.168
B	Normal stem	1.153

synthate containing the tracer isotope C^{13} did not move in appreciable amounts through areas of the stem in which the living cells had been killed by the hot wax treatment. The very slight excess of the fraction from below the killed section over the ratio for normal non-fed stems might indicate that a trace passed through the killed tissue, but the excess is almost insignificant, since normal non-fed stems were found to have ratios which varied as much as the ratios in question.

The completeness of the blockage to upward movement is further illustrated by plants 5 and 6. Both tips were normal in appearance and were receiving an adequate water supply. If one-half of one per cent as much new photosynthate had gone to the tip of plant 6 as went to the tip of plant 5 the mass ratio would have been 1.185, a value outside the experimental error. Hence, it is likely that less than this amount of new organic material crossed the barrier. This sets the upper limit of transport in the transpiration stream of a killed stem at less than 1 per cent of the total in a normal stem.

Results of simultaneous transport experiments with radioactive phosphorus and heavy carbon.—Although it was concluded from the fact that the bean plant lived and carried on photosynthesis for as long as 14 days after a 3 cm. section of its stem had been killed with hot wax, that the killed section still allowed the passage of water, it seemed desirable to see whether an inorganic tracer such as radioactive phosphate might traverse the killed section while the leaves were being exposed to enriched CO_2 . Accordingly, 5 cc. of a solution containing 355 mgm. of Na_2HPO_4 in 50 cc. of water and originally containing 6 microcuries of radioactivity, but which was well decayed at the time of its use, were used to cover the roots of a bean plant which was simultaneously using $C^{13}O_2$ in photosynthesis. After the selected tissues were burned in the Van Slyke apparatus for C^{13} analysis, the inorganic residue remaining in the combustion fluid was analyzed for radioactivity with a sensitive Geiger counter and a Herbach-Rademann amplifier. All combustion fluids were diluted to 10 cc. Exactly 3.32 cc. of each solution were used in the counter tube. The total counts were allowed to accumulate over a period of such length as to make the probable error less than 1 per cent. According to

Barnes and Salley (1943) the probable error for any period is defined as:

$$\pm 0.67 \sqrt{\frac{\text{total number of observed counts for the period.}}{}}$$

To make an error of less than 1 per cent, more than 4500 counts must be accumulated. Since these accumulated counts are relative to an ever-present background count, one of the latter counts was made with distilled water or with normal combustion fluid each day. The results of simultaneous feeding experiments performed with a normal plant, number 7, and a plant with a section of killed stem, number 8, are shown in figure 6. When cor-

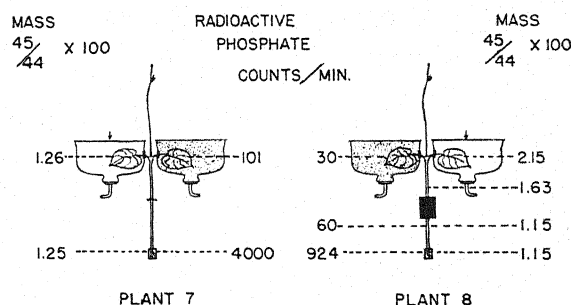


Fig. 6. Results of simultaneous transport experiments using radioactive phosphorus and heavy carbon. The CO_2 mass $45/44 \times 100$ ratios are expressed in the outside columns, while the results of the Geiger counter determinations are shown in the center columns. Radioactivity measurements were made on the illuminated leaf material rather than on non-illuminated leaf material as the above diagram indicates. These Geiger counter determinations are all single determinations, uncorrected for a background count of 15 counts per min. The CO_2 ratio analyses are means of at least two analyses and are relative to a ratio of 1.16 for normal plant tissue. Non-illuminated leaves are stippled areas. Killed stem section is area in black.

rected for a background of 15 counts per minute, it is found that the concentration of P^{32} in the leaf of plant 7 is 2.1 per cent of that in the root while the leaf in plant 8 had a concentration of 1.6 per cent of that in the root. Hence the transport of inorganic phosphorus has been retarded very little by the injury which completely blocked the downward movement of organic matter.

Experiments on cross transport to the opposite primary leaf.—While carrying out the preceding experiments, it was noticed that under the conditions in which one primary leaf was exposed to light and to enriched CO_2 , the opposite primary leaf did not accumulate any of the heavy isotope. This lack of cross transport suggested the following study: Samples were obtained from the leaves of plants after one leaf of each plant had been exposed to light and to enriched CO_2 . In the first experiments, the C^{12} leaf (as the leaf which was not exposed to the enriched CO_2 is designated), was exposed to light and to CO_2 of ordinary isotopic ratio. When results did not indicate cross transport of C^{13} into the C^{12}

leaf, the feeding of ordinary CO_2 to the C^{12} leaf was discontinued, and the leaf kept in the dark with no added CO_2 . While most of the ratio analyses were made on CO_2 from the combustion of whole leaf tissue, in later experiments, the CO_2 from the combustion of the soluble carbohydrate extract (80 per cent alcohol) of the petiole and the main veins of the leaf was analyzed. Bean plants were used which had both normal and killed stems. Some experiments were made on plants from which the stem tips had been removed as in plant 2 in figure 2. The results in table 3 show that the iso-

the C^{12} leaf and had been exported again before the time of the final ratio analysis of the leaf tissue, an extension of experiment 6 in table 3 was made in which a small leaf punch was taken from the C^{12} leaf at intervals and burned for analysis of CO_2 for C^{13} .

The results in table 4 show that at no time was there any excess of the C^{13} in the unfed leaf. The deviations from the means of the above ratios are about the usual variation observed when low-ratio samples are measured with the mass spectrometer on successive days. Anatomical studies of the bean

TABLE 3. Results of the studies of cross transport to the opposite primary leaf. Group A includes normal-stem plants. The C^{12} leaf in experiments 1-3 was exposed to light and to CO_2 of the ordinary isotopic ratio. In all further experiments this leaf was not exposed to added CO_2 and was kept in the dark. Group B is composed of killed-stem bean plants. In experiments 8-11 the plants had killed stem sections below the primary leaf node, such as plant 5, fig. 5. In experiment 12 is a plant with a killed stem section above the primary leaf node such as plant 6, fig. 5. In experiment 13 is a plant with killed stem sections both above and below the primary leaf node such as plant 4, fig. 5. Group C includes normal-stem bean plants similar to Group A, the difference being that while the ratio analyses were made on CO_2 samples obtained directly from the combustion of leaf sections in Groups A and B, the ratio analyses for Group C were made on the soluble carbohydrate extract as described in the text. Ratios are the means of at least two determinations and are relative to a normal tissue ratio of 1.16. \downarrow indicates that the leaf was illuminated.

Group	Experiment number	Mass $45/44 \times 100$.		Millimoles		Sample	Duration of experiment
		C^{13} leaf	C^{12} leaf	C^{13}O_2 fed	C^{12}O_2 fed		
A	1	4.00	1.18	1.50 \downarrow	1.50 \downarrow	leaf tissue	24 hours
	2	3.70	1.16	2.20 \downarrow	2.50 \downarrow	tissue	30 hours
	3	3.90	1.15	3.20 \downarrow	2.20 \downarrow	tissue	34 hours
	4	2.05	1.16	1.27 \downarrow	0.00	tissue	24 hours
	5*	3.33	1.15	1.10 \downarrow	0.00	tissue	24 hours
	6	3.70	1.15	1.70 \downarrow	0.00	tissue	43 hours
	7	2.52	1.16	1.12 \downarrow	0.00	tissue	114 hours
B	8	2.15	1.15	0.85 \downarrow	0.00	tissue	35 hours
	9	3.20	1.16	1.20 \downarrow	0.00	tissue	32 hours
	10*	3.30	1.17	1.48 \downarrow	0.00	tissue	24 hours
	11	4.20	1.25	0.98 \downarrow	0.00	tissue	24 hours
	12	3.31	1.15	1.10 \downarrow	0.00	tissue	22 hours
C	13	3.18	1.15	1.34 \downarrow	0.00	tissue	48 hours
	14	3.57	1.18	1.27 \downarrow	0.00	soluble	24 hours
	15	1.34	1.16	0.95 \downarrow	0.00	carbohydrate extract	24 hours

* Indicates bean plant without stem tip as plant 2, fig. 2.

topic ratio of CO_2 in the C^{12} leaf is unaffected by (1) the presence or absence of light and ordinary CO_2 in the C^{12} leaf chamber, (2) the amount of enriched CO_2 fed the C^{13} leaf, (3) the duration of the experiments, (4) the presence or absence of stem tips and, (5) killing the stem tissue with hot wax. In no case, except one, was there any suggestion of transport of tagged photosynthate to the opposite primary leaf. This is in sharp contrast with the ready transport of the labeled photosynthate to other parts of the plant, as observed in the previous experiments. In one experiment (no. 11) which did give slight positive evidence of cross transport, there may have been an error in manipulation.

To determine whether or not C^{13} had reached

plant by Doult (1932) gave evidence of vascular connections between the opposite sides of the stem at the primary leaf node. In view of the crossing over and anastomosing of vascular tissues, some direct transport of heavy carbon into the opposite primary leaf might be expected.

In order to ascertain whether or not the opposite side of the stem contained significantly less heavy carbon than the side adjacent to the C^{13} fed leaf, the normal stem from experiment 6, table 3, was divided in the vicinity of the primary leaf node into two equal longitudinal halves, the plane of division being perpendicular to the primary leaf petioles. Sections of these halves were burned to CO_2 for isotopic analysis. These results are shown in figure 7. The general equal lateral distribution

TABLE 4. *Ratio analyses of leaf punches taken from the C¹² leaf at intervals during which time the opposite primary leaf was being exposed to light and to CO₂ enriched with C¹³. Between sampling the C¹² leaf was kept in the dark and was not fed added CO₂. Each ratio is the mean of the indicated number of determinations and is relative to a normal bean tissue isotopic ratio of 1.16 ± 0.01.*

Time	Number of determinations	Mass 45/44 × 100	Maximum variation from the mean
0 hours	2	1.149	±0.005
10 hours	4	1.152	±0.018
21 hours	3	1.157	±0.004
28 hours	3	1.141	±0.005
43 hours	3	1.151	±0.014

of the heavy carbon isotope in the stem makes it all the more surprising that none of it could be detected in the unfed leaf.

Results of starvation experiments which further demonstrate the lack of cross transport between opposite primary leaves.—Two bean plants were selected and one leaf of each plant was placed between sheets of porous black paper. The sheets were held together by paper clips so that the leaves were in the dark without being deprived of ready exchange of gases. This arrangement also allowed for the observation of the leaves at intervals. Plant 1 was placed in the dark and plant 2 was placed in the light. The experiment was terminated after 220 hours, at which time observations on the darkened leaves showed marked starvation symptoms accompanied by wilting and discoloration. Leaf punches were made and dried overnight at 100°C. The data are presented in table 5. Both leaves of the darkened plant were discolored and wilted, as was the covered leaf of the illuminated plant. In

rapidly growing regions would have opportunity to accumulate a greater amount of heavy carbon materials than would the older tissues. Analysis of

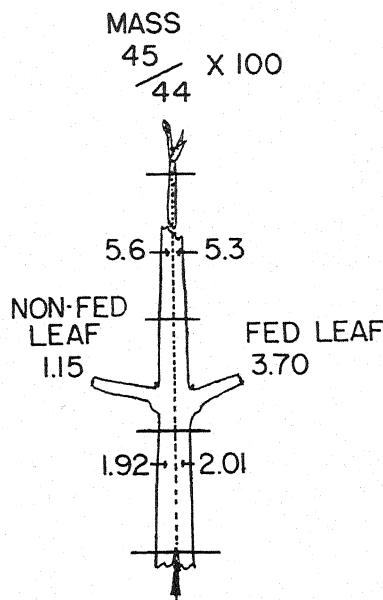


Fig. 7. Ratio analyses of carbon dioxide obtained by the combustion of the proximal and distal halves of the stem with regard to the leaf fed with C¹³. The plane of division is along the dotted line and vertical to the plane of the paper. Each figure is the mean of at least two determinations and is relative to the isotopic ratio of 1.16 for normal leaf tissue. Ratio of CO₂ fed to right primary leaf was 10.2.

sections of stem above the primary leaf node showed a correlation between maximum elongation and the amount of heavy carbon fixed by the stem. The highest concentration of tracer carbon was found

TABLE 5. *Data on the starvation of leaves in darkness. Column 2, dry weights of the leaf punches. Columns 3 and 4 maximum width of leaves. Column 5 condition of the leaves.*

Description	Dry weight leaf punch mgm/cm ²	Original leaf width	Final leaf width	Condition of leaf
Plant 1 after 220 hours in darkness				
Covered leaf	0.9	9 cm.	9 cm.	Wilted, discolored
Uncovered leaf	0.7	9 cm.	9 cm.	Wilted, discolored
Plant 2 after 220 hours in light				
Covered leaf	0.8	6 cm.	6 cm.	Wilted, discolored
Uncovered leaf	1.3	6 cm.	10 cm.	Green, fresh

the case of the latter plant, this demonstrates a lack of cross transport of food from the uncovered leaf, which increased in size and weight as a result of photosynthesis.

DISCUSSION.—Detection of greater concentrations of C¹³ in the stem tips and in the root tips than in the older stem and root tissue shortly after a photosynthetic period during which C¹³ labeled CO₂ was fixed is according to expectation, since the

in the apical centimeter of the stem. Under the conditions of the experiment, neither the stem tip nor the opposite primary leaf were necessary for the conduction of compounds labeled with C¹³ from the primary leaf to the roots of the bean plant.

Analysis of the proximal and distal halves of the bean stem with relation to the fed primary leaf failed to show any difference in the lateral distribution of heavy carbon in the stem tissue. The rea-

son for the lack of transport of C^{13} compounds into the opposite primary leaf is not apparent from this work. In view of the anastomosing of vascular tissue in the region of the primary leaf node and the crossing over of vascular traces as reported by Harris *et al.* (1921), and by Douth (1932), one would expect cross transport of materials from one primary leaf to the other.

SUMMARY

Methods are described for the incorporation of CO_2 enriched with the tracer isotope C^{13} into plant tissue by photosynthesis, and also for sampling plant tissue, burning tissue samples to CO_2 , and analyzing this gas for the presence of the tracer element.

Rapid transport of labeled photosynthate upward and downward to metabolically active regions such as the root tip and stem tip is shown.

A correlation is shown between the ratio excess of C^{13} and the rapidity of growth in the region of the stem tip.

Radioactive phosphorus passed through killed areas of bean stems while compounds containing C^{13} did not.

The failure to find that any C^{13} tagged material moved upward or downward through stems killed with hot wax suggests that the exclusive path of transport of these materials is in the living tissues, probably the phloem.

Lack of cross transport of labeled photosynthate to the opposite primary leaf is shown under conditions of light or darkness, feeding or starvation.

Equal lateral distribution of new photosynthate is found in the stem of plants fed on only one side.

DEPARTMENT OF BOTANY,
UNIVERSITY OF MINNESOTA,
MINNEAPOLIS, MINNESOTA

LITERATURE CITED

- BARNES, R. B., AND D. J. SALLEY. 1943. Analysis for potassium by its natural radioactivity. Industrial and Engineering Chemistry, Analytical Edition. 15: 4-7.
- BEBER, J. 1937. The role of chlorophyll in photosynthesis. Ph.D. thesis, University of Minnesota.
- BELKENGREN, R. O. 1941. The use of the heavy carbon isotope as a "tracer" in plant metabolism. Ph.D. thesis, University of Minnesota.
- BIDDULPH, O. 1941. Diurnal migration of injected radiophosphorus from bean leaves. Amer. Jour. Bot. 28: 348-352.
- , AND JANE MARKLE. 1944. Translocation of radiophosphorus in the phloem of the cotton plant. Amer. Jour. Bot. 31: 65-70.
- BOTH, M. P. 1937. Stoffwanderung in einfachen Systemen. Rec. Trav. Bot. Néerl. 34: 1-68.
- CARPENTER, T. M. 1923. An apparatus for the exact analysis of air in metabolism investigations with respiratory exchange chambers. Jour. of Metabolic Res. 4: 1-25.
- COLWELL, R. N. 1942. The use of radioactive phosphorus in translocation studies. Amer. Jour. Bot. 29: 798-807.
- CRAFTS, A. S. 1938. Translocation in plants. Plant. Physiol. 13: 791-814.
- . 1939. The movement of viruses, auxins, and chemical indicators in plants. Botanical Review 5: 471-504.
- CURTIS, O. F. 1935. The translocation of solutes in plants. McGraw-Hill Book Company, New York.
- DIXON, H. H. 1938. Transport of substances in plants. (Croonian Lecture.) Bot. School of Trinity College, Dublin Notes 4: 279-303.
- DOUTH, MARGARET T. 1932. The anatomy of *Phaseolus vulgaris* L. var. Black Valentine. Michigan Agric. Exper. Sta. Bull. 128.
- GUSTAFSON, F. G. 1939. Upward transport of minerals through the phloem of stems. Science 90: 306-307.
- , AND MARJORIE DAKEN. 1937. Further evidence for the upward transport of minerals through the phloem of stems. Amer. Jour. Bot. 24: 615-621.
- HAMM, P., R. B. HARVEY, AND E. S. MILLER. 1941. Quantitative spectroscopic analysis of stem "tracheal" fluids for inorganic constituents. Proc. Soc. Exp. Biol. and Med. 46: 347-351.
- HARRIS, J. A., E. W. SINNOTT, J. Y. PENNYPACKER, AND G. B. DURHAM. 1921. The vascular anatomy of dimerous and trimerous seedlings of *Phaseolus vulgaris* L. Amer. Jour. Bot. 8: 63-102.
- HEVESY, G. 1923. The absorption and translocation of lead in plants. Biochem. Jour. 17: 439-445.
- MASON, T. G., AND E. PHILLIS. 1937. The migration of solutes. Bot. Rev. 3: 47-71.
- MILLER, E. S. AND G. O. BURR. 1935. Carbon dioxide balance at high light intensities. Plant Physiol. 10: 73-92.
- MÜNCH, ERNST. 1930. Stoffbewegungen in der Pflanze. Gustav Fischer. Jena.
- NIER, A. O. 1940. A mass spectrometer for routine isotope abundance measurements. Rev. of Sci. Instruments 11: 212-216.
- , AND J. BARDEEN. 1941. The production of concentrated carbon (13) by thermal diffusion. Jour. of Chem. Physics 9: 690-692.
- , AND E. A. GULBRANSEN. 1939. Variations in the relative abundance of the carbon isotopes. Jour. Amer. Chem. Soc. 61: 697-698.
- OVERSTREET, E., AND T. C. BROYER. 1940. The nature of the absorption of radioactive isotopes by living tissues as illustrated by experiments with barley plants. Proc. Nat. Acad. Sci. U.S.A. 26: 16-24.
- OVERTON, J. B. 1911. Studies on the relation of the living cells to transpiration and sap-flow in *Cyperus*. Bot. Gaz. 51: 28-63.
- STOUT, P. R., AND D. R. HOAGLAND. 1939. Upward and lateral movement of salts in certain plants as indicated by radioactive isotopes of potassium, sodium and phosphorus absorbed by roots. Amer. Jour. Bot. 26: 320-324.
- VAN NIEL, C. B., S. RUBEN, S. F. CARSON, M. D. KAMEN, AND J. W. FOSTER. 1942. Radioactive carbon as an indicator of carbon dioxide utilization. VIII. The role of carbon dioxide in cellular metabolism. Proc. Nat. Acad. Sci. U.S.A. 28: 8-15.
- VAN SLYKE, D. D., AND J. FOLCH. 1940. Manometric carbon determination. Jour. Biol. Chem. 136: 509-541.
- WOLKOFF, I. 1918. The effect of ammonium sulfate in nutrient solution on the growth of soybeans in sand cultures. Soil Sci. 5: 123-150.

GROWTH IN VITRO OF EXCISED TOBACCO AND SUNFLOWER TISSUE WITH DIFFERENT TEMPERATURES, HYDROGEN-ION CONCENTRATIONS AND AMOUNTS OF SUGAR ¹

Albert C. Hildebrandt, A. J. Riker, and B. M. Duggar²

FACTORS RESPONSIBLE for the initiation and continuation of pathological growth have been studied with plant materials because they offer certain opportunities for research (Riker, 1939) not provided by animal materials. Some advantages of plant materials include (a) large numbers, (b) low cost, (c) ease of experimental use, (d) physiological variability, (e) genetic purity, and (f) the possibility of tissue culture on a medium of known composition (Riker, 1942). Among the plant materials the crown gall disease caused by *Phytoplasma tumefaciens* (Smith and Town.) Bergey *et al.* has received special attention because of certain similarities between this pathological growth in plants and cancer of higher animals (e.g., Smith, 1922; Levine, 1936; White and Braun, 1942). The early literature on crown gall was reviewed by Riker and Bergey (1935). The anatomy of the host tissue and physiology of the bacteria have been extensively studied by various investigators. The physiology of the host tissue, however, has remained relatively obscure because of certain technical difficulties, some of which may be overcome by cultivation of tissue on a synthetic medium *in vitro*.

Plant tissue culture has been accomplished only recently. The idea was probably first suggested by Haberlandt (1902). Limited growth of excised root tips was reported by Kotte (1922) and by Robbins (1922), while unlimited growth was reported by White (1934) with tomato roots. Since root tips contain several kinds of tissue, their cultivation may be considered organ culture. True tissue cultures capable of unlimited growth were first reported by White (1939) with tobacco callus and by Gautheret (1939) and Nobecourt (1939) with carrot callus. Recently, White and Braun (1941) have cultivated tissue from "secondary" crown galls on sunflower. The extensive history and applications of plant tissue culture have been reviewed by White (1943).

In tissue culture simple, somatic cells may be isolated from the influence of neighboring cells, tissues, and organs and may be studied under controlled conditions on a synthetic medium containing only ingredients whose chemical formulae can be written. Such a technique offers a tool for the study of critical vital processes of tissue metabolism. While many basic requirements of excised root tips *in vitro* have been determined by various investi-

gators (White, 1943), the optimum conditions for growth of true plant tissue cultures have been hardly touched. Therefore, before more complex studies of tissue metabolism were examined some of the basic physical and physical-chemical requirements of certain plant tissue cultures were studied.

The present paper (abstracted, Hildebrandt, Riker and Duggar, 1944a) reports the effects of different temperatures, hydrogen-ion concentrations, and amounts of sucrose on growth *in vitro* of excised tobacco and sunflower tissue. In a later paper (abstracted, Hildebrandt, Riker and Duggar, 1944b) the influence of the composition of the medium will be reported in detail.

MATERIALS AND METHODS.—Two kinds of tissue were used in these investigations, both of which can grow indefinitely *in vitro*. The tobacco tissue was from the interspecific hybrid *Nicotiana glauca* Grah. ♀ × *N. langsdorffii* Weinm. ♂ (Kostoff, 1930; Levine, 1937) and was originally isolated and supplied by Dr. P. R. White. The other tissue was isolated from "secondary," petiolar crown galls on sunflower (*Helianthus annuus* L. var. Giant Russian), and, as explained later, was free from the crown-gall bacteria. The method involved removing gall-bearing petioles from the plant, stripping back the epidermis over the gall, and with a sterile scalpel removing portions of the enlarged tissue to the basic agar medium. This was accomplished aseptically and rapidly to avoid excessive injury, desiccation, and chance contamination of the tissue. No disinfectant was used. In figure 1 are illustrated the tobacco and sunflower tissues as they appear in culture.

A certain percentage of such gall tissues when originally isolated always contained crown-gall bacteria, which eventually multiplied, escaped from the tissue, and grew over the medium and explant. In one set of isolations tissue cultures were started from fifty "secondary," petiolar crown galls. Sixteen of these original isolates contained crown-gall bacteria and were necessarily discarded. None of the remaining isolates produced bacteria after the first subculture. The stock cultures of the sunflower tissue were developed from tissue from one "secondary," petiolar crown gall. It had been subcultured by subdividing many times at monthly intervals since December, 1941, on a medium suitable for growth of both excised plant tissue and crown-gall bacteria. Since no bacteria appeared during three years, the tissue was considered free from crown-gall bacteria.

The stock cultures of tobacco and sunflower tissue were maintained in diffuse light at laboratory temperatures. The basic medium was the mineral salt, sucrose, vitamin B₁, glycine, agar medium de-

¹ Received for publication March 5, 1945.

This work was supported in part by the International Cancer Research Foundation and the Wisconsin Alumni Research Foundation. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

² The writers are indebted to Eugene Herrling for preparing the illustrations.

veloped by White (1942). The medium as used in these studies contained the following quantities of salts in milligrams per liter of distilled water: 360 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 200 $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 200 Na_2SO_4 , 80 KNO_3 , 65 KCl , 16.5 $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 2.5 $\text{Fe}_2(\text{SO}_4)_3 \cdot 6\text{H}_2\text{O}$, 4.5 $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 1.5

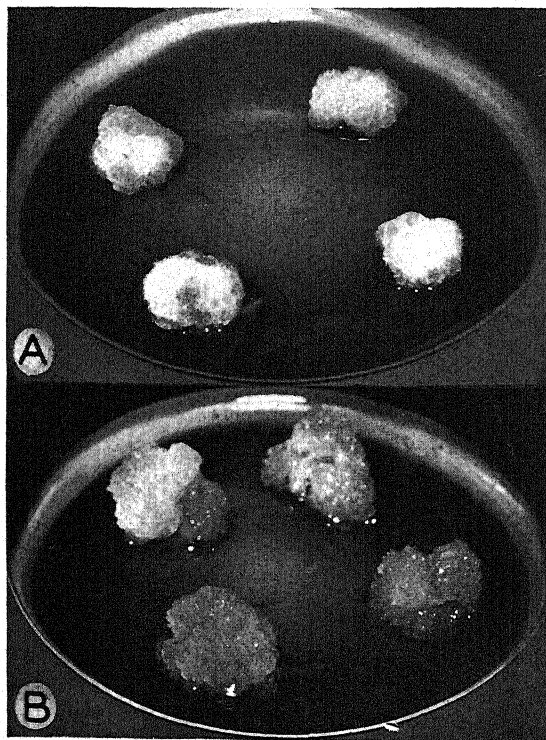


Fig. 1. A. Excised tobacco callus tissue after six weeks on agar medium in a 125 ml. Erlenmeyer flask. B. As A, except sunflower tissue.

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 H_3BO_3 , and 0.75 KI . It contained in addition 20 grams sucrose, 7.5 grams agar, and, respectively, 3.0 and 0.1 milligrams per liter of glycine and thiamine hydrochloride. The chemicals were all of reagent grade except sucrose, boric acid, and potassium iodide. A record was kept of the source and impurities of the constituents, but it is omitted here to conserve space. The sucrose was added as cane sugar. The agar was either the flake or shredded type and was leached by washing in distilled water at least three times daily over a three- to four-day period. The medium was prepared from concentrated stock solutions as described by White (1943). All experiments were carried out in 125 milliliter Erlenmeyer flasks each containing 50 milliliters of medium. The flasks had been washed previously in potassium dichromate-sulfuric acid cleaning solution and rinsed in tap and distilled water.

Subcultures were made by transferring stock tissue to a sterile Petri dish, by cutting it with a sterile scalpel into irregular hexahedral pieces 3 millimeters in greatest dimension, and by transfer-

ring four good tissue pieces to each culture flask. The seed tissue pieces each weighed 20 to 30 milligrams. In experiments involving a number of different treatments the seed tissue pieces from each stock tissue piece were distributed equally among the different treatments. All operations were carried out under aseptic conditions in a special transfer room. Cheesecloth-covered cotton plugs were used on all flasks, and these were finally covered with waxed paper to prevent excessive desiccation of the medium. All experimental cultures, unless otherwise noted, were incubated in a special culture room in the dark at $26^\circ \pm 1^\circ\text{C}$. A favorable humidity and a circulation of air were maintained by passing air from a fan over an open dish of water. Six flasks each containing four tissue pieces were used per treatment, and each experiment was repeated at least three times at different times of the year, unless otherwise noted. After six-weeks incubation, the tissues were removed from the culture flasks and weighed individually.

VARIABILITY OF THE TISSUE.—Of the various methods available for measuring growth, the final wet weight of the tissues was chosen. As with most biological materials, considerable variation occurred between tissues grown under similar conditions. Therefore, the results of individual experiments were analyzed statistically to determine any significance between treatments. The data from a single representative experiment are presented in table 1, and an analysis of variance of these data is presented in table 2. The data are from an experiment carried out from December 16, 1943, to January 28, 1944, on the effect of sucrose concentration on growth *in vitro* of excised tobacco tissue. The total wet weights of four tissues per flask are given so that with six flasks there was a total of six "replicates" for each sugar concentration. There was one set with no sugar, and there were eight sets with different concentrations. Experiments showed that there was variation between cultures grown at different times, and, therefore, control cultures on the basic medium were always run with individual experiments and each experiment analyzed statistically. To verify results from individual experiments, statistical analyses were made also on combined results from similar experiments repeated at different times considering each time as one replicate.

The analysis of variance of this representative experiment (table 1) gave an F value for between treatments of 34.63 (table 2) which is highly significant. The mean significant difference between treatments for this experiment was, respectively, 0.06 and 0.08 at the 5 and 1 per cent levels. The differences between averages of treatments were significant at the 5 per cent level at all sugar concentrations except between 0.5 and 1 per cent and between 2 and 4 per cent (table 1). It should be restated that these average values are from one representative experiment. Data averaged from three

TABLE 1. Total wet weight of tobacco tissue grown on the basic medium with different sucrose concentrations.

Sucrose concentration	"Replicates" ^a						Total
Per cent	1	2	3	4	5	6	
0	.14	.09	.11	.12	.09	.12	.67 (.11) ^b
.125	.31	.33	.34	.38	.32	.26	1.84 (.31)
.25	.36	.32	.38	.37	.51	.30	2.24 (.37)
.5	.33	.43	.53	.41	.43	.47	2.60 (.43)
1.0	.58	.44	.37	.47	.44	.42	2.72 (.45)
2.0	.37	.46	.35	.38	.39	.36	2.31 (.39)
4.0	.37	.27	.39	.30	.44	.35	2.12 (.35)
8.0	.20	.23	.28	.27	.26	.22	1.46 (.24)
16.0	.10	.11	.12	.15	.13	.15	.76 (.13)
Total	2.76	2.68	2.77	2.85	3.01	2.65	16.72

^a Each number represents the total wet weight in grams of four tissues from one culture flask.

^b Average wet weight of four tissues per flask in parentheses.

such experiments are presented under the "Effect of Sucrose Concentration."

EXPERIMENTAL RESULTS.—*Influence of temperature.*—Tobacco and sunflower tissues were cultured on the basic medium at temperatures ranging from 4° to 37°C. Some variation existed in light intensity and humidity at the different temperatures. Observations with stock cultures maintained under

TABLE 2. Analysis of variance on data from table 1.^a

Source of variance	Degrees of freedom	Sums of squares	Mean square	F	F at the one per cent level
Between treatments.	8	.7480	.0935	34.63	2.93
Error	45	.1222	.0027
Total	53	.8702

^a The authors are grateful to Dr. J. H. Torrie, Assistant Professor of Agronomy, and Virginia B. Beal, Assistant in Agricultural Statistical Service for their assistance in this phase of the problem.

light and dark conditions showed that light had little effect. Excessive drying of the media was avoided by putting wax paper over the cotton plugs and by keeping pans of water in incubators warmer than room temperature.

The influence of temperature on the growth of these two tissues is shown in figure 2. Each point on the curve is the average wet weight of 72 tissues

from three experiments carried out May 5 to June 21, 1943, January 21 to March 3, 1944, and March 17 to April 30, 1944. The optimum range for tobacco tissue was about 26° to 32°C., and that for sunflower about 24° to 28°C. These results agree with Skoog's (1944) report that growth of tobacco tissue in liquid media increased with increasing temperature from 5° to 33°C. The differences between

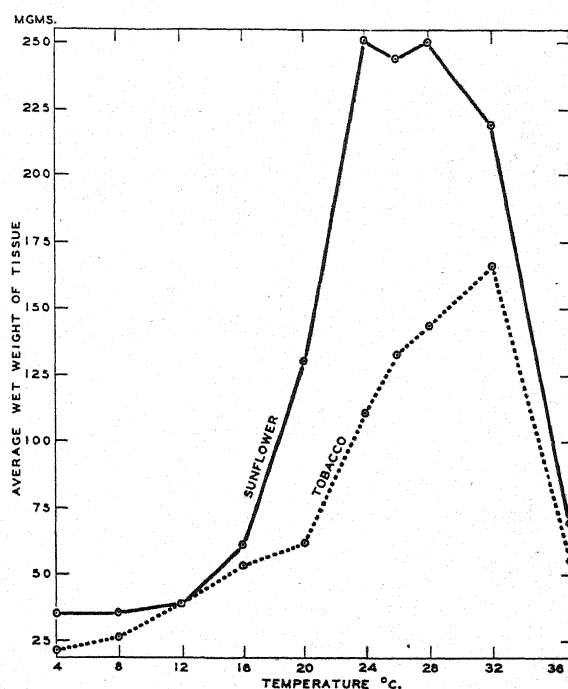


Fig. 2. Effects of temperature on the growth of excised sunflower and tobacco tissues growing *in vitro* on the basic medium.

treatments were highly significant in all six experiments as determined by statistical analyses of each of the individual experiments.

The effect of cool temperature was to slow up growth. Thus, after six weeks certain tissues maintained at 8°C. were removed to the laboratory at room temperature where they soon resumed the usual rate of growth. White (1937) showed that tomato roots will also endure low temperatures for long periods of time, growing only slowly but resuming a normal rate when removed to favorable temperatures.

Effect of hydrogen-ion concentration.—The importance of the hydrogen-ion concentration was likewise observed on growth of both sunflower and tobacco tissue. They were cultured on media with original hydrogen-ion concentrations ranging, respectively, from pH 2.0 to 9.9 and from 2.5 to 9.9. The acidities were obtained by adding aseptically predetermined amounts of 0.1N NaOH or 0.2N HCl to the basic medium before it solidified. No special attempt was made to buffer the media at the various acidities. The original and final pH

measurements of the media, as well as the final pH of the tissue, were determined with a Coleman glass electrode pH meter. The pH of the tissue was determined after macerating it in a small volume of distilled water.

The average wet weight and the pH of the tissue from such experiments carried out at three different times of the year are presented in figure 3. To secure points on the curves (figure 3) for weight *vs.* original and *vs.* final pH of the medium the final wet weights of the tissues were used for both curves. For example, in figure 3A (weight *vs.* original pH) each point represents the average weight of tissues from culture flasks with original acidities ranging, respectively, from pH 2.0 to 2.4, 2.5 to 2.9, 3.0 to 3.4 . . . 9.5 to 9.9. Because of the drift in the pH of the medium, the points on one curve were usually based on a different number of tissues than corresponding points on the other.

Both tissues grew within the range pH 3.5 to 7.9. Best growth of tobacco tissue occurred on media with original values averaging from pH 5.0 to 5.4 and final values averaging from pH 5.5 to 5.9. The average pH of the tissues on the latter media was 6.0. The pH of the tobacco tissue in cultures with final values of pH 7.5 and 8.4 was not determined. Best growth of sunflower tissue occurred on media with original values averaging from pH 5.5 to 5.9

and final values averaging from pH 6.0 to 6.4. The average pH of the tissues on the latter media was 5.9.

The pH of the medium drifted from the extremes toward the center during the six-week culture period. This drift in reaction was probably influenced on the alkaline side by metabolic and atmospheric carbon dioxide and on the acid side by the utilization of some of the nitrates in the potassium and calcium salts.

Effect of sucrose concentration.—Cultures of both tissues were made on the basic medium with sucrose concentrations from 0 to 160 grams per liter. The sucrose was added as cane sugar. The average final wet weights of eighty tissue pieces for sunflower and eighty-eight tissue pieces for tobacco from experiments carried out at two and three different times respectively are presented in figure 4. From these data it may be seen that both tissues grew on media containing 1.25 to 80 grams per liter of sucrose. They grew well within the range 5 to 20 grams per liter, with the optimum for both species at 10 grams per liter. Differences between concentrations were significant at the 1 per cent level (table 1) in all experiments for both species. The average weights given for zero sucrose concentration and for 16 per cent (figure 4) represent approximately those of the original seed tissue pieces.

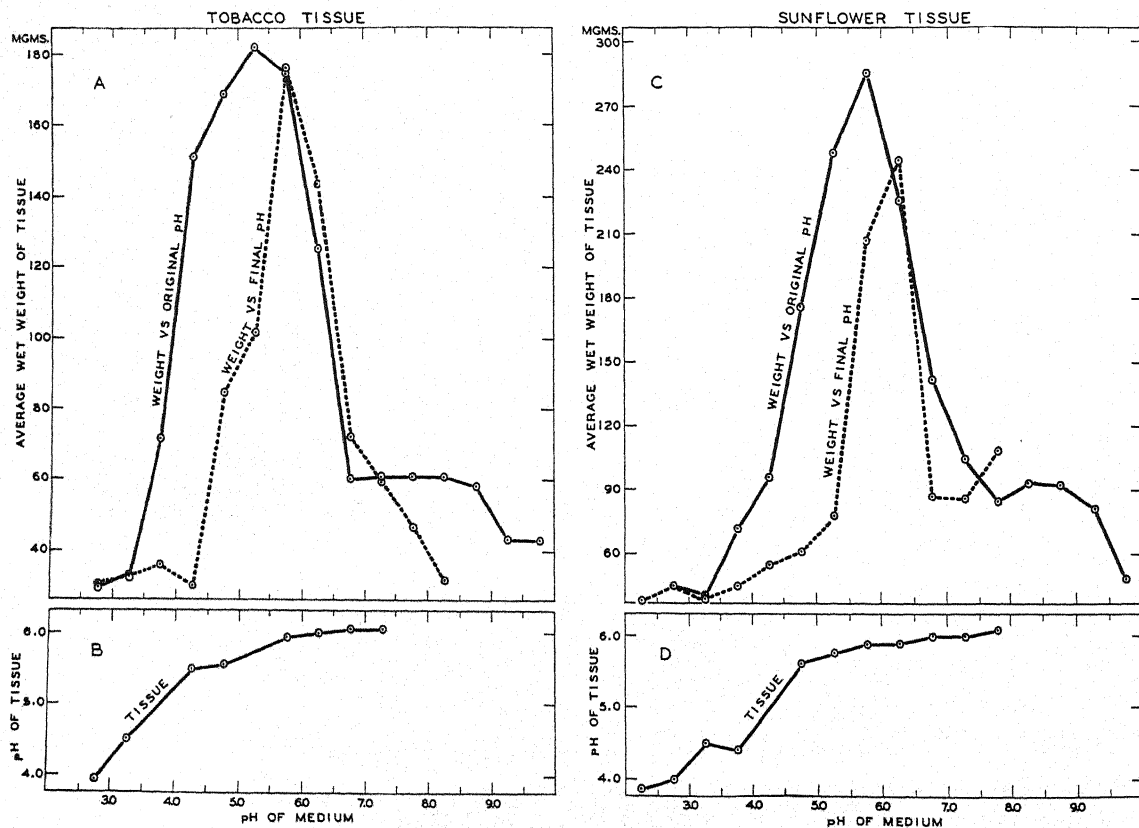


Fig. 3. Effects of the pH of the medium on the weight of excised tobacco and sunflower tissues growing *in vitro*, and on the pH of the tissue.—A. Weight of tobacco tissue compared with original and final pH of the basal medium.—B. Final pH of the tissue.—C. As A except for sunflower tissue.—D. As B except for sunflower tissue.

Thus, there was little or no growth of the tissues at these two concentrations. The final pH of the media from 0 to 160 grams per liter for sunflower, respectively, averaged pH 6.0, 5.8, 5.8, 5.9, 5.8, 5.8, 5.8, 5.7, and 5.5 and for tobacco 6.1, 6.2, 6.3, 6.2,

in temperature, acidity, and sugar concentration affect the growth *in vitro* of these two tissues. Correspondingly, they provide an improved foundation for critical studies with this technique of tissue metabolism.

SUMMARY

Certain environmental factors influence the growth of excised tobacco and sunflower callus tissue *in vitro*. When both tissues were cultured over the range 4° to 37°C., the optimum temperature range for tobacco tissue was about 26° to 32°C., and that for sunflower tissue was about 24° to 28°C.

Best growth of tobacco tissue occurred on media with original acidities averaging from pH 5.0 to 5.4 and final acidities from pH 5.5 to 5.9. Sunflower tissues grew best on media with original acidities averaging from pH 5.5 to 5.9 and final acidities from pH 6.0 to 6.4.

When progressively increasing amounts of sucrose were added, respectively, to different lots of the basic medium, both species grew best with a 1 per cent concentration and did well from 0.5 to 2 per cent. There was some growth at 4 and 8 per cent, but no growth at 16 per cent or on media lacking sugar.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN

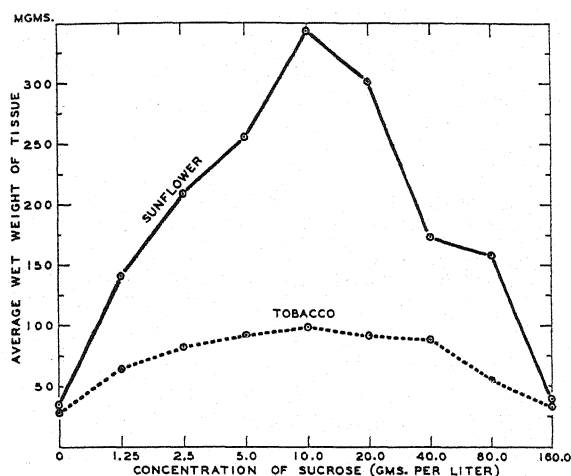


Fig. 4. Effect of various sucrose concentrations on the growth of excised sunflower and tobacco tissue *in vitro*.

6.1, 6.1, 6.0, 5.9. These acidities are close to the optimum for growth of both tissues (figure 3).

These results indicate how much certain changes

LITERATURE CITED

- GAUTHERET, R. 1939. Sur la possibilité de réaliser la culture indéfinie des tissus de tubercules de carotte. *Compt. Rend. Acad. Sci., Paris* 208:118-121.
- HABERLANDT, G. 1902. Kulturversuche mit isolierten Pflanzenzellen. *Sitzungsb. Akad. Wiss. Wien, Math.-Natur. Kl.* 111:69-92.
- HILDEBRANDT, ALBERT C., A. J. RIKER, AND B. M. DUGGAR. 1944a. Effect of temperature, pH, and sucrose concentration on growth of excised tobacco and sunflower tissue *in vitro*. *Amer. Jour. Bot.* 31:10s. (Abstract.)
- , ———, AND ———. 1944b. Effect of crown-gall bacterial metabolites, crown-gall tissue extracts, and the composition of the medium on growth *in vitro* of excised tobacco and sunflower tissue. *Phytopath.* 34:1003-1004. (Abstract.)
- KOSTOFF, D. 1930. Tumors and other malformations on certain *Nicotiana* hybrids. *Zentralbl. Bakt. Parasit, Infect.* 2 Abt. 81:244-260.
- KOTTE, W. 1922. Wurzelmeristem in Gewebekulture. *Ber. Deut. Bot. Ges.* 40:269-272.
- LEVINE, M. 1936. Plant tumors and their relation to cancer. *Bot. Rev.* 2:439-455.
- . 1937. Tumors of tobacco hybrids. *Amer. Jour. Bot.* 24:250-256.
- NOBECOURT, P. 1939. Sur la pérennité et l'augmentation de volume des cultures de tissus végétaux. *Compt. Rend. Soc. Biol., Paris* 130:1270-1271.
- RIKER, A. J. 1939. Physiological relations between host and parasite in crown gall. An example of basic biological research with plant materials. *Amer. Jour. Bot.* 26:159-162.
- . 1942. The relation of some chemical and physico-chemical factors to the initiation of pathological plant growth. *Growth* 6:105-117.
- , AND T. O. BERGE. 1935. Atypical and pathological multiplication of cells approached through studies on crown gall. *Amer. Jour. Cancer* 25:310-357.
- ROBBINS, W. J. 1922. Cultivation of excised root tips and stem tips under sterile conditions. *Bot. Gaz.* 73:376-390.
- SKOOG, FOLKE. 1944. Growth and organ formation in tobacco tissue culture. *Amer. Jour. Bot.* 31:19-24.
- SMITH, E. F. 1922. Appositional growth in crown-gall tumors and in cancers. *Jour. Cancer Res.* 7:1-49.
- WHITE, P. R. 1934. Potentially unlimited growth of excised root tips in a liquid medium. *Plant Physiol.* 9:585-600.
- . 1937. Survival of isolated tomato roots at sub-optimal and supraoptimal temperatures. *Plant Physiol.* 12:771-776.
- . 1939. Potentially unlimited growth of excised plant callus in an artificial nutrient. *Amer. Jour. Bot.* 26:59-64.
- . 1942. Plant tissue cultures. *Ann. Rev. Biochem.* 11:615-628.
- . 1943. A handbook of plant tissue culture. Lancaster, Pa.
- , AND A. C. BRAUN. 1941. Crown-gall production by bacteria-free tumor tissue. *Science* 94:239-241.
- , AND ———. 1942. A cancerous neoplasm of plants: Autonomous bacteria-free crown-gall tissue. *Cancer Res.* 2:597-617.

BRAZILIAN CHYTRIDS. VI. RHOPALOPHLYCTIS AND CHYTRIOMYCES, TWO NEW CHITINOPHYLLIC OPERCULATE GENERA¹

John S. Karling

FRESH-WATER chytrids which inhabit the chitinous exuviae of insects are fairly common in nature, but until recently they have not received much attention from mycologists. The first study devoted exclusively to these saprophytes was made in 1903 by Petersen in Denmark, and it was not until 1937 that they were again given serious consideration. In the latter year Sparrow (1937) recorded the occurrence of Petersen's Danish species in the New World and described several new American species from the exuviae of caddisflies, midges, and of other insects, the larval stages of which develop in fresh water. Since that time, several additional species have been reported by the author (1944) from the Amazon Valley in Brazil, which indicates that chitinophyllic chytrids are widespread in nature and more abundant than has been generally realized.

Such chytrids grow well on the chitinous skeletons of insects and appear to derive most of their energy from chitin. These unusual growth requirements suggested to the author, particularly after Miss Anne Hanson, a graduate student at Columbia University, had found that some species can be grown successfully on thin strips of shrimp shell, that insect-inhabiting chytrids might be grown on artificial chitinous media. Although such chytrids may be readily trapped and isolated on shrimp shells, this type of bait may also become heavily infected with other aquatic fungi and protozoa and is not wholly satisfactory. However, if all but the pure chitin is removed, better results may be obtained. In purifying the chitin, the procedures of Benecke (1905), Folpmers (1921), Johnson (1932), Benton (1934), Zobel and Rittenberg (1938), and Hock (1940) were used. Shrimp, crab, and lobster shells were scrubbed clean of dirt and decalcified in cold one per cent hydrochloric acid which was changed several times in the course of a week. The material was then washed in water and soaked in two per cent potassium hydroxide for ten days to remove the protein and other organic matter except chitin. Following this treatment, the limp leathery shells were boiled in several changes of ethyl alcohol for three days or until no solid matter was obtained on evaporation of a portion of the extract, after which the shells were dried and stored for future use. The treatment with alkali and alcohol removes all or most of the pigment, leaving the chitin greyish-white or semi-transparent. The ventral scales of the shrimp's tail are usually quite thin and almost transparent, and when treated in the manner described above and cut into thin strips, make a satisfactory substratum for trapping, growing, and studying mi-

croscopically certain chitinophyllic chytrids. Up to the present time species of *Rhizidium*, *Obelidium*, *Asterophlyctis*, *Siphonaria*, *Rhopalophlyctis*, *Chytriomycetes*, *Rhizoclostridium*, and *Polychytrium* have been isolated from fresh-water cultures in Brazil and the U.S.A. on bits of pure chitin.

Processed shrimp, lobster, and crab shells may be ground in a powder mill and added directly to nutrient agar, but this does not make a very satisfactory medium. Chitin is difficult to pulverize in a mill, and the fragments usually obtained are so large that they sink to the bottom of an agar plate and are not directly available to monocentric chytrids sown on the surface. In the case of polycentric species like *Polychytrium aggregatum* (Ajello, 1942), however, good results may be obtained because the rhizomycelium grows downward in the agar and soon spreads from one bit of chitin to another.

A more satisfactory and highly dispersed chitin for inclusion in agar media may be obtained by dissolving chitin in a 1:1 solution of sulfuric acid overnight in the refrigerator and then precipitating with a twenty per cent potassium hydroxide solution. The milky-white precipitate is then filtered and washed with distilled water on a Büchner funnel until the extract shows neutral with litmus. The flocculated chitin may be added in varying amounts to plain or enriched agar as desired, autoclaved, and poured. The flask, however, should be shaken carefully to disperse the chitin evenly before pouring. Chitin-agar media prepared in this fashion are usually milky-white in appearance. Chitinophyllic chytrids planted on such media digest the chitin as the rhizoids and rhizomycelium spread, and as a result a clear zone develops around each thallus or colony within a few days.

The present contribution concerns the structure and life cycles of two new chitinophyllic operculate genera which were isolated on chitin and grown in the manner described above. These fungi were first found on unidentified insects in fresh water in Brazil and later collected in abundance on the exuviae of mayflies in Connecticut, New York, and Virginia, U.S.A. The first of these genera is characterized by predominantly clavate, septate or continuous, extramatrix sporangia, an intramatrix bushy tuft of thread-like rhizoids, and by posteriorly uniflagellate zoospores which swarm in a vesicle outside of the sporangium. Because of the characteristic shape of its sporangia and its occurrence on exuviae of insects, the name *Rhopalophlyctis sarcoptoides* is proposed for this fungus. The second genus includes two species and is distinguished by extramatrix sporangia, extensive intramatrix rhizoids which arise from the base of the sporangium or apophysis, posteriorly uniflagellate zoospores which swarm in a vesicle outside

¹ Received for publication March 1, 1945.

This work has been greatly facilitated by a Grant-in-Aid of Research from the Graduate Faculties of Columbia University.

the sporangium, and by extramatrical resting spores. Except for its operculate sporangia, this genus is almost identical with species of *Rhizidium* (Karling, 1944) and *Phlyctochytrium*. For this new genus the author proposes the name *Chytriomyces* because of its characteristic *Chytridium*-like thallus. The two fungi for which this genus is established are similar in structure and development but differ in pigmentation. One species is characterized by a conspicuous golden-red, refringent globule in the zoospore and by reddish-brown resting spores, and is accordingly named *C. aureus*. The other species is distinguished by a hyaline refractive globule in the zoospore and is called *C. hyalinus*.

Chytriomyces n. gen.—Thalli monocentrici, eucarpici; sporangiis et sporis perdurantibus extramatriculibus, apophysis et rhizoideis intramatriculibus. Sporangia operculata. Zoosporae a posteriore uniflagellatae. Sporae perdurantes germinantes ut prosperangia, parte anteriore emergente ut zoosporangis membranum tenui ad superficiem sporae.

Thalli monocentric, eucarpic, consisting of an extramatrical sporangium or resting spore and an intramatrical apophysis and rhizoidal system. Sporangia operculate. Apophysis usually present; system of rhizoids usually arising from opposite sides of apophysis, tapering abruptly, branched and extensive. Zoospores posteriorly uniflagellate; emerging from sporangium and swarming on the outside in a vesicle. Resting spores extramatrical to substratum, functioning as prosperangia in germination.

C. aureus n. sp.—Fungus saprophyticus. Sporangii luteo-rubris, laevibus, globosis et 8–40 μ , vel leviter ovalibus et 10–20 \times 12–23 μ ; operculo apicali aut sub apicali, 4–6 μ diam. Zoosporae ovals, 3–3.5 \times 5 μ , globulo refractivo luteo-rubro, 1.5–2 μ diam.; flagello 22–25 μ longo. Apophysis sphaerica aut sub sphaerica, 3–6 μ diam.; sporis perdurantibus sphaericis et 6–20 μ , aut ovalibus et 6–10 \times 12–16 μ ; parietibus crassiusculis, 2 μ , luteo-fuscis, laevibus; germinantes ut prosperangia, parte anteriore emergente ut zoosporangio membranum tenui ad superficiem sporae.

Sporangia golden-red, smooth, spherical (8–40 μ), or slightly oval (10–20 \times 12–23 μ); operculum apical or subapical shallow saucer-shaped, 4–6 μ in diam. Zoospores oval, 3–3.5 \times 5 μ , with a golden-red refractive globule, 1.5–2 μ in diam.; flagellum 22–25 μ long; zoospores emerging and swarming in vesicle 2–32 minutes before breaking out and swimming away; vesicle continuous with interior of sporangium. Apophysis, when present, spherical to subspherical, 3–6 μ in diam. Resting spores spherical (6–20 μ), oval (6–10 μ \times 12–16 μ), with a thick (2 μ), golden-brown smooth wall and numerous closely packed granules or globules; functioning as prosperangia in germination.

Saprophytic in exuviae of mayflies and on chitin, Flores Nabuco near Manaus, Amazonas, Brazil; Sharon, Connecticut; Brewster, New York; and Mt. Prospect, Virginia, U.S.A.

C. hyalinus n. sp.—Fungus saprophyticus. Spo-

rangii hyalinis, laevibus, globosis et 10–60 μ ; operculo apicali aut sub apicali, 8–16 μ diam. Zoosporae ovals, 3–3.5 \times 5–5.5 μ , globulo refractivo hyalino, 1–1.5 μ diam., flagello 18–20 μ longo. Apophysis sphaerica, subsphaerica, fusiformis, 3–7 μ diam. Sporae perdurantes laeves, sphaericae (10–20 μ), ovals (6–8 \times 10–14 μ), elongatae, pyriformes, clavatae, leviter irregulares, parietibus crassiusculis, 2 μ , subfuscis; germinantes ut prosperangia, parte anteriore emergente ut zoosporangio membranum tenui ad superficiem sporae.

Sporangia hyaline, smooth, usually spherical, 10–60 μ ; operculum apical or subapical, shallow, saucer-shaped, 8–16 μ in diam. Zoospores oval, 3–3.5 μ \times 5–5.5 μ , with a small (1–1.5 μ), hyaline refractive globule; flagellum 18–20 μ long; zoospores emerging and swarming in vesicle, 1–16 minutes before breaking out and swimming away; vesicle continuous with interior of sporangium. Apophysis when present spherical, subspherical, fusiform, or elongate, 3–7 μ in diam. Rhizoidal system well developed, main axis up to 7 μ in diam.; extending for a distance of 300 μ . Resting spores spherical (10–20 μ), oval (6–8 \times 10–14 μ), elongate, clavate, pyriform, or slightly irregular, with a smooth, thick (2 μ), light-brown wall; containing a large central refractive globule surrounded by a few to several smaller ones; functioning as prosperangia in germination.

Saprophytic on exuviae of mayflies and on bits of chitin, Flores Nabuco near Manaus, Amazonas, Brazil; Sharon, Connecticut; Brewster, New York; and Mt. Prospect, Virginia, U.S.A.

Rhopalophlyctis n. gen.—Thallus monocentricus, eucarpicus. Sporangia extramatricalia; rhizoideis intramatriculibus. Sporangia operculata, stipitata aut sessilia, septata aut eseptata. Zoosporis a posteriore uniflagellatis. Sporae perdurantes non visae.

Thallus monocentric, eucarpic, consisting of an extramatrical sporangium and an intramatrical rhizoidal system. Sporangia operculate, stalked or sessile, septate or continuous. Zoospores posteriorly uniflagellate, emerging from sporangium and swarming in a vesicle. Resting spores unknown.

R. sarcotoides n. sp.—Fungus saprophyticus. Sporangia hyalina, laevia, obpyriformia (10–70 \times 15–90 μ), clavata (12–40 \times 25–180 μ), aut sphaerica (8–40 μ), septata aut eseptata, sessilia aut stipitata; operculo 6–12 μ diam. Zoosporae ovals, 5 \times 6.5–7 μ ; flagello 25–28 μ longo. Sporae perdurantes non visae.

Thalli numerous, up to 500 on one host. Sporangia hyaline, smooth with fairly thick (1.5 μ) walls, obpyriform (10–70 \times 15–90 μ), clavate (12–40 \times 35–180 μ), spherical (8–40 μ), or elongate, continuous or septate, sessile or stalked, base of stalk sometimes expanded irregularly to form a foot; operculum shallow, saucer-shaped, 6–12 μ in diam. Zoospores oval, 5 \times 6.5–7.5 μ , with a refractive globule 3 μ in diam. near posterior end; flagellum 25–28 μ long; zoospores swarming in a hyaline vesicle out-

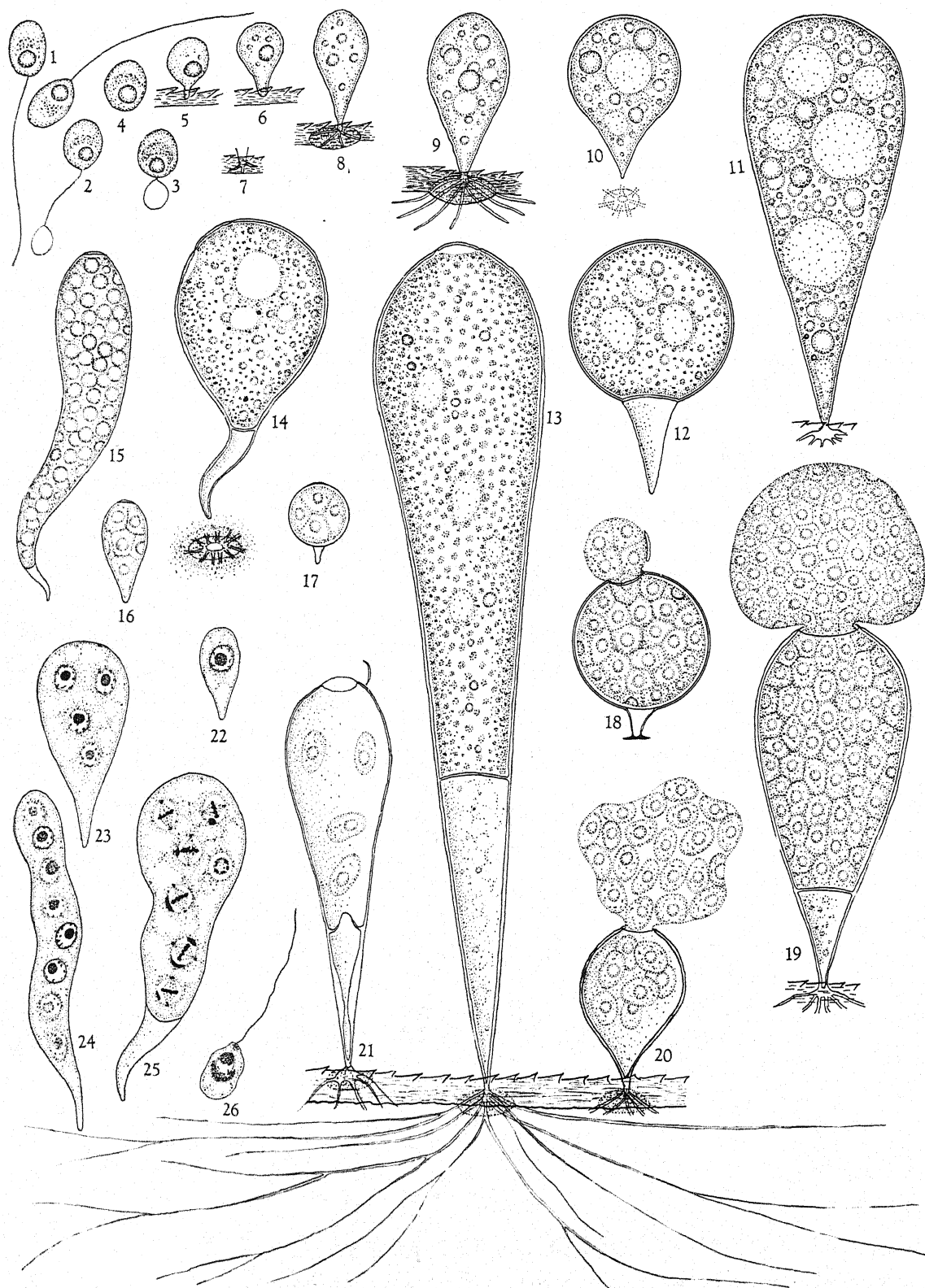


Fig. 1-26. *Rhopalophlyctis sarcoptoides*.—Fig. 1. Zoospores. $\times 1400$.—Fig. 2, 3. Absorption of flagellum. $\times 1400$.—Fig. 4. Zoospore at rest. $\times 1400$.—Fig. 5-8. Germination of zoospore and development of basal peg and rhizoids. $\times 1400$.—Fig. 9. Rhizoids entering the lumen of insect case. $\times 1300$.—Fig. 10. Small sporangium which has been

side of sporangium for 2–8 minutes before breaking out and swimming away; vesicle continuous with interior of sporangium. Rhizoids arising as a tuft of fine threads from base of thallus and extending for a distance of 200 μ ; threads simple or branched. Resting spores unknown.

Saprophytic on exuviae of mayflies, Flores Nabuco near Manaus, Amazonas, Brazil; Sharon, Connecticut; Brewster, New York; and Mt. Prospect, Virginia, U.S.A.

Life cycle of Rhopalophlyctis sarcoptoides.—As was noted earlier, a few specimens of this species were first observed on an unidentified insect in Brazil and later found in abundance on the exuviae of mayflies near Pawling, New York, and Sharon, Connecticut, U.S.A. In these last two localities as many as 500 thalli were often found on one insect case. With such material as an inoculum, the author was able to transfer the fungus to pure chitin and chitinagar. However, growth on these media was only sparse, and the results so far obtained have not been very satisfactory.

The zoospores of *R. sarcoptoides* are oval in shape and contain a large hyaline refractive globule (fig. 1). As the close of the motile period approaches, a loop or vesicle is formed at the end of the flagellum (fig. 2) and becomes larger (fig. 3) as the flagellum shortens. The flagellum thus appears to be absorbed by the zoospore as in *Rhizidium verrucosum* (Karling, 1944). After the zoospore has come to rest (fig. 4) on the insect case, it forms a short germ tube or peg which penetrates the integument (fig. 5). The diameter and length of the peg vary considerably (fig. 5, 6), and these variations often determine whether the sporangia will be stalked or sessile. Short branches soon develop from the base of the peg (fig. 7) and grow radially between the layers of the integument (fig. 8) for a short distance and finally enter the lumen of the insect case (fig. 9). These branches are the rudiments of the thread-like rhizoids which continue to elongate, branch, and eventually attain the proportions shown in figure 13. The tip of the peg sometimes enlarges slightly as the rhizoids develop, and takes on the appearance of a small irregular foot (fig. 11, 19–21). Quite often the sporangia appear to have a large circular foot (fig. 8–10, 14), but this seems to be an illusion. As the radially oriented rhizoids grow out from the peg, they separate the layers of the integument in a circular area, which often has the appearance of a circular holdfast at the base of the thallus. Study of fixed and stained longitudinal sections of the thalli and integument have not revealed the presence of

such a foot or holdfast. The thalli may be readily pulled off (fig. 10, 14), leaving a small hole in the integument; were a special holdfast present, it is not likely that the thalli could be removed so readily. Occasionally, however, removal is not easy, and a part of the integument may adhere to the base of the stalk (fig. 18) when the thallus is pulled off.

The successive developmental stages of the sporangium are shown in figures 5 to 15, and it is obvious that the internal protoplasmic changes involved are similar to those of other rhizidiaceous chytrids. As the incipient sporangium enlarges, the refractive globules increase in size and number and usually become as numerous and large as those shown in figure 11. At the same time the protoplasm becomes vacuolate. This phase is followed by a gradual dispersal of the refractive material (fig. 12) until the protoplasm becomes coarsely granular in appearance (fig. 13). After an interval of several hours the process appears to be reversed whereby the refractive material aggregates again (fig. 14) to form the globules of the definitive zoospores (fig. 15). In elongate and large sporangia the protoplasm often becomes highly vacuolate and sparse in the basal portion. Such a state is usually followed by septation in which the basal part is delimited from the apical portion (fig. 12–15, 18, 19, 21). The position of the septum in the sporangium varies, as is well shown in these figures, and the cross wall may sometimes be curved or irregular (fig. 25, 21). In a few instances it was found to be greatly thickened, and the thickening often extended to the lateral walls of the basal portion (fig. 21).

As was noted in the diagnosis above, the sporangia of *R. sarcoptoides* vary markedly in size. Sporangia up to 180 μ long are not uncommon, and minute sporangia with only eight (fig. 16) or four (fig. 17) zoospores sometimes occur. The sporangia are uninucleate in the early developmental stages (fig. 22, 24) but become multinucleate as they increase in size (fig. 23, 24). Nuclear division is distinctly mitotic with an intranuclear spindle and large nucleole (fig. 25). The chromosomes are small in size and usually so closely crowded that it is difficult to determine their number. In a few cases six to eight discrete bodies have been counted in polar views.

When the zoospores are mature, the operculum is pushed off, and the spores ooze out slowly in a spherical mass (fig. 18). The mass enlarges (fig. 19) as additional zoospores emerge and soon becomes enveloped by a thin hyaline membrane as described by the author (1944) for species of *Rhizidium*. However, if the dehiscence of the sporangium is

pulled off, leaving a small hole in the integument. $\times 1300$.—Fig. 11. Clavate sporangium filled with large refractive globules, vacuoles, and granular cytoplasm. $\times 1200$.—Fig. 12. Beginning of granular stage. $\times 1200$.—Fig. 13. Mature thallus consisting of clavate, septate, extramatrical sporangium with granular protoplasm and intramatrical branched rhizoids. $\times 1200$.—Fig. 14. Stalked globular sporangium with subapical operculum. $\times 1200$.—Fig. 15. Clavate sporangium shortly before cleavage. $\times 1200$.—Fig. 16–17. Minute 8- and 4-spored sporangia. $\times 1200$.—Fig. 18–20. Emergence of zoospores from sporangium and their swarming in a vesicle. $\times 1200$.—Fig. 21. Empty septate sporangium with thickened basal walls. $\times 1000$.—Fig. 22, 23, 24. Uni-, tetra-, and 8-nucleate sporangia. $\times 1400$.—Fig. 25. Simultaneous nuclear division in sporangium. $\times 1400$.—Fig. 26. Zoospore fixed and stained *in toto* with nucleus, nucleole and nuclear cap. $\times 1500$.

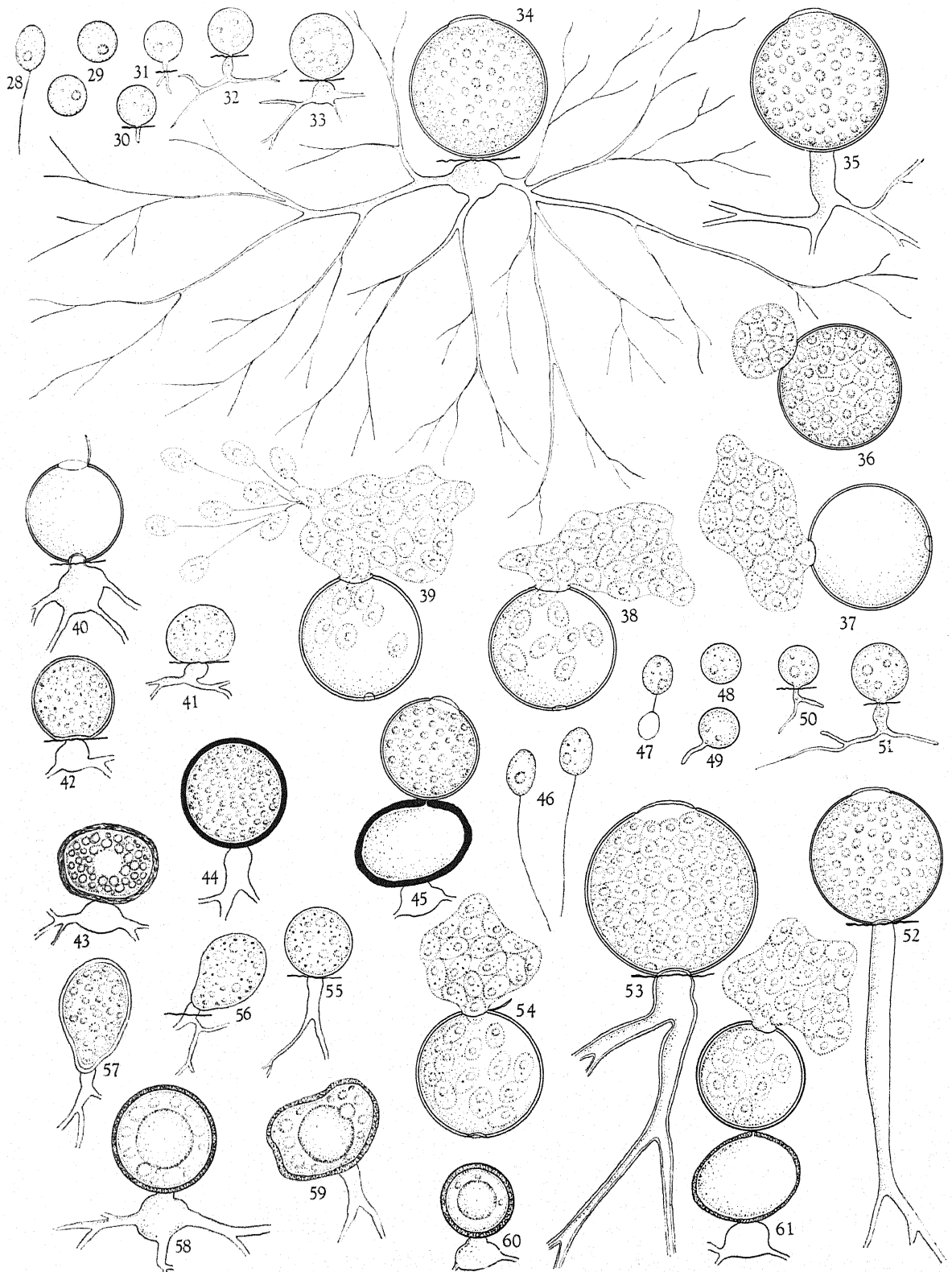


Fig. 28-45, *Chytridiomyces aureus*; fig. 46-61, *Chytridiomyces hyalinus*.—Fig. 28. Oval motile zoospore. $\times 1400$.—Fig. 29. Spores after coming to rest. $\times 1400$.—Fig. 30-33. Germination stages and young thalli. $\times 1400$.—Fig. 34. Mature apophysate thallus. $\times 1200$.—Fig. 35. Mature non-apophysate thallus. $\times 1200$.—Fig. 36-39. Successive stages in

premature or abnormal, no membrane develops, and the zoospores gradually separate without becoming actively motile. Under normal conditions the zoospores swarm violently in the vesicle from two to eight minutes before rupturing it and escaping. The vesicle is continuous with the interior of the sporangium, and the zoospores pass readily from one structure to the other.

Resting spores have not been found in *R. sarcoptoides*, so that the taxonomic position and relationships of this species are uncertain. Its clavate, elongate, continuous or septate, operculate sporangia are very similar to those of *Cylindrochytridium johnstonii* (Karling, 1941), and on this basis the two species appear to be closely related. It is to be noted, however, that the latter species is usually apophysate and intramatrical, while *R. sarcoptoides* is largely extramatrical and nonapophysate. Furthermore, in *R. sarcoptoides* the zoospore gives rise directly to the sporangium, while in *C. johnstonii* the sporangium develops intramatrically from the germ tube.

Life cycles of Chytrimyces aureus and C. hyalinus.—These species appear to be more common in nature than *R. sarcoptoides* and have been collected in great abundance in several localities in Brazil, and in Connecticut, New York, New Jersey, and Virginia, U.S.A. In cases of infection of exuviae of mayflies, the sporangia were only rarely found on the exterior surface, and in almost all instances they projected into the lumen of the empty larval cases. They were, nevertheless, extramatrical to the substratum, whereas the apophysis, when present, and rhizoids were intramatrical in the chitinous layers of the integument. This space relation of the chytrid to its substratum is probably due to more favorable nutritional conditions inside the larval cases. As the mayflies emerge, they probably leave behind body fluids and other substances which are favorable to the growth of chytrids. Free-swimming zoospores then enter the open exuviae and germinate, with the result that most thalli occur within the insect case. However, when water cultures with infected exuviae are baited with thin strips of pure chitin, the latter become heavily infected with thalli the sporangia of which are all on the surface. In some instances small bits of chitin were almost completely covered by golden-red and hyaline sporangia which were often so closely crowded that they appeared angular in cross section. Both species grew well on soft 0.5 per cent chitin agar and completed their vegetative phase in from four to six days. A few thalli of both species have been grown to maturity on thin bits of onion skin also, which indicates that they are not completely dependent on chitin.

Except for the formation of an operculum, the development of *C. aureus* and *C. hyalinus* is so similar to that of *Rhizidium brazilienses*, *R. laeves* (Karling, 1944), and certain species of *Phlyctochytrium* and *Rhizophidium* that a detailed description is unnecessary. Figures 28 to 45 illustrate the life cycle of *C. aureus*, and figures 46 to 61 relate to *C. hyalinus*. In *C. aureus* the zoospore with its brilliant golden-red globule (fig. 18) comes to rest (fig. 29) and forms a germ tube which penetrates the substratum (fig. 30). The germ tube soon branches (fig. 31), and the rudiments of the rhizoids are thus laid down at an early stage. Branching is often dichotomous, and when only two main branches are formed, they frequently diverge in almost opposite directions (fig. 32) in the thin skeleton of mayflies. In the event that the thallus is to become apophysate, a localized swelling in the branched germ tube soon begins to form. It may occur above (fig. 32) or at the point of branching (fig. 33). In the former event, the rhizoids of the resultant mature thallus will be centered on the base of the apophysis, whereas in the latter case they will appear to have arisen from the sides as well as the base (fig. 33, 34). The disposition of the rhizoids relative to the apophysis thus depends to a large extent on the region of origin of the apophysis.

After the rudiments of the rhizoids and apophysis have been established, the extramatrical spore body begins to enlarge (fig. 32, 33) and eventually develops into the mature sporangium. During the early developmental stages, the refractive globule of the zoospore appears to fragment, and loses its brilliant golden-red color. Other less brilliant minute granules soon appear (fig. 32, 33), and by the time the sporangia are half grown, the cytoplasm is only faintly orange-red in color. Then as the refractive material aggregates to form the definitive globules of the zoospores, the sporangia attain their characteristic color. These changes in pigmentation are very similar to those described by Sparrow (1937) for the developmental stages of *Siphonaria variabilis*. A mature apophysate thallus with extensive rhizoids is shown in figure 34. The latter structures do not branch as richly as those of species of *Phlyctochytrium* and *Rhizophidium*, but taper more abruptly and soon run out to fine filaments like the rhizoids of *Siphonaria* and *Rhizidium*. In *S. aureus* they do not extend much beyond a radius of 150 μ and rarely attain a diameter of more than 5 μ at the point of origin. Although the majority of thalli of this species are apophysate, non-apophysate ones are not uncommon, particularly on purified chitin and chitin agar (fig. 35). In *C. hyalinus* non-apophysate thalli are even more common (fig. 52, 53, 55, 59). The

the emergence and swarming of the zoospores from a free-floating sporangium. $\times 1200$.—Fig. 40. Empty sporangium with attached operculum. $\times 1200$.—Fig. 41-44. Stages in development of resting spores. $\times 1200$.—Fig. 45. Germination of a resting spore. $\times 1200$.—Fig. 46. Oval motile zoospores. $\times 1400$.—Fig. 47. Vesiculation and absorption of flagellum. $\times 1400$.—Fig. 48. Spore at rest. $\times 1400$.—Fig. 49-51. Germination stages and young thallus. $\times 1400$.—Fig. 52. Non-apophysate sporangium with a long rhizoid axis. $\times 1200$.—Fig. 53. Similar sporangium shortly before emergence of the zoospores. $\times 1200$.—Fig. 54. Free-floating sporangium with swarming zoospores. $\times 1200$.—Fig. 55-60. Stages in development of resting spores. $\times 1200$.—Fig. 61. Germinated resting spore. $\times 1200$.

presence or absence of an apophysis, therefore, does not appear to be a fundamental distinctive character of *Chytriumyces*. The author has found this to be true also of other chitinophyllic genera such as *Siphonaria* and *Rhizidium*. As was noted earlier, the apophysate thalli are very similar in appearance to those of *Phlyctochytrium* except for the presence of an operculum, while the non-apophysate ones often resemble those of *Rhizophydium*.

The mature sporangia of *C. aureus* and *C. hyalinus* are readily broken off from the apophysis and rhizoids during the process of mounting for microscopic study and float free in the surrounding water. Under such conditions they do not degenerate but shed their zoospores in the normal manner (fig. 36-39, 54). Such sporangia are ideal for study of their dehiscence and the emergence and swarming of the zoospores. As the latter emerge, the low inconspicuous operculum is pushed off to one side of the sporangium and usually disappears from sight. The zoospores ooze out slowly (fig. 36), form a globular mass at the exit orifice, and lie quiescent for twenty to sixty-five seconds. During this period the globular mass apparently becomes enveloped by a thin hyaline membrane. The zoospores soon begin to move and jerk about slightly, and within a few seconds active swimming begins. As in the case of *Rhizidium* and *Rhopalophylyctis* the zoospores swarm so rapidly and violently that the surrounding membrane or vesicle is constantly modified in shape as the zoospores dash against its surface (fig. 37-39, 54). In the case of free-floating sporangia, the sporangium itself may often be moved about by the violence of the spores. It thus becomes obvious that the vesicle with its zoospores is attached to the sporangium and is continuous with its interior. This continuity is clearly evident as some zoospores dash back into the sporangium from the vesicle and out again. The period of swarming lasts from two to thirty-two minutes after which the vesicle ruptures at one or several places, and the zoospores escape (fig. 39). The length of the swarming period apparently depends on the strength of the vesicular membrane and the ease with which it is ruptured. In many cases of prolonged swarming, the membrane fails to rupture, and the zoospores consequently come to rest within it. The above-described emergence and behavior of the zoospores appear to be normal for *C. aureus* and *C. hyalinus*. If, however, the sporangia delisce prematurely or too late, or if conditions are otherwise abnormal, no vesicle is formed, and the zoospores separate without becoming actively motile and eventually disintegrate.

The development of the thallus and the behavior of the zoospores of *C. hyalinus* are so similar to those of *C. aureus* that nothing further need be said about them. It is sufficient to note that the zoospore contains a hyaline refringent globule instead of a golden-red one as in *C. aureus*. Furthermore, the thalli are more commonly non-apophysate and often attain greater size. Sporangia up to 70 μ in diameter

are not uncommon, and in cases of non-apophysate thalli, the main rhizoidal axis may be up to 7 μ in diameter and fairly thick-walled (fig. 53) with its branches extending over a radius of 300 μ .

As far as is known at present, resting spore development in both species is similar to that of the sporangia up to a certain stage. The first visible difference is the accumulation of refractive granular and globular material in the cytoplasm (fig. 41, 55, 56), and as the incipient spore attains definitive size its wall begins to thicken (fig. 42, 57). In *C. aureus* the wall becomes dark reddish-brown in color (fig. 43-45) and 1.8 to 2.4 μ in thickness. The refractive material increases in amount, in the meantime, so that by the time the spore is mature, its interior is filled with more or less uniformly coarse granules (fig. 43, 44), which are often evenly dispersed around a central vacuole (fig. 43). The majority of spores in this species are oval in shape (fig. 43, 45), although spherical ones occasionally occur. In *C. hyalinus*, on the other hand, the spores vary from spherical (fig. 58, 60), oval (fig. 61), and pyriform (fig. 57) to irregular in shape with a hyaline to light brown wall 1.5-2.5 μ thick. Furthermore, the accumulated refractive globules coalesce (fig. 58, 59) to form a large hyaline central one (fig. 60). Coalescence of globules is often so complete that the cytoplasm appears optically hyaline and structureless except for the large suspended central globule. In cases of incomplete fusion, a few small globules may surround the larger one. So far, no conclusive evidence of fusion of thalli has been found in resting spore formation in either species.

The resting spores of both species germinate under laboratory conditions after a dormant period of from two to three months. In germinating, they function as prosperangia and give rise to a thin-walled operculate sporangium (fig. 45, 61). The zoospores from such sporangia swarm in a vesicle like those from the primary sporangia described above.

The relationships of *Chytriumyces* are not certain at present. As was pointed out previously, this genus has the same type of development and appearance as species of *Rhizidium*, *Phlyctochytrium*, and *Rhizophydium*, but the presence of an operculum precludes close relationship with these genera. Among the known monocentric operculate chytrids, *Chytriumyces* is most similar to *Catenochytridium*, a genus which Miss Berdan (1939) described as having extramatrical operculate sporangia and extramatrical resting spores; the latter function as prosperangia in germination. In a more intensive study, however, Miss Berdan (1941) later found that the resting spores were formed intramatrically from the primary apophysate cell. In *Chytriumyces*, on the other hand, the resting spores are extramatrical and develop directly from the zoospore. *Catenochytridium*, furthermore, is always apophysate, and in *C. carolinianum* the apophysis consists of a complex linear catenoidal series of globular segments. In *C. laterale*

(Hanson, 1944), on the other hand, a large primary apophysate cell predominates, which indicates perhaps a trend toward or away from a simple apophysis like that of many thalli of *Chytriomycetes*. As was noted earlier, the presence of an apophysis does not appear to be generically significant in *Chytriomycetes*, so that this character is not a sound basis of comparison. The apophysate thalli of *Chytriomycetes* are also very similar to those of *Amphicypellus*, a new chytrid genus recently described by Ingold (1944) from Great Britain. This apophysate chytrid is extramatrical except for the tips of the rhizoid branches, and since Ingold is not sure of the presence of an operculum, very little can be said at present about the relation of *Amphicypellus* to *Chytriomycetes*.

SUMMARY

Rhopalophlyctis and *Chytriomycetes* are two new monocentric operculate genera which were first found on unidentified insect skeletons in fresh water in Brazil and later collected in abundance on the

exuviae of mayflies in Connecticut, New York, and Virginia, U.S.A. Both genera are chitinophyllic and have been isolated and grown on thin strips of pure chitin and soft chitin agar.

Rhopalophlyctis includes a single species, *R. sarcopoides*, and is characterized by extramatrical predominantly clavate, septate or continuous operculate sporangia, an intramatrical bushy tuft of thread-like rhizoids, and by posteriorly uniflagellate zoospores which swarm in a vesicle outside of the sporangium. *Chytriomycetes* includes two species, *C. aureus* and *C. hyalinus*, and is distinguished by extramatrical globular operculate sporangia, intramatrical apophyses and rhizoids, posteriorly uniflagellate zoospores which swarm in a vesicle outside of the sporangium, and by extramatrical resting spores which function as prosperangia in germination.

DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY,
NEW YORK 27, NEW YORK

LITERATURE CITED

- AJELLO, L. 1942. *Polychytrium aggregatum*, a new cladochytriacous genus. *Mycologia* 34:442-451.
- BENECKE, W. 1905. Über *Bacillus chitinovor*, einen Chitinzersetzenden Spaltpilz. *Bot. Zeit.* 63:227-231.
- BENTON, ANNA G. 1935. Chitinovorous bacteria. *Jour. Bact.* 29:449-463.
- BERDAN, H. 1939. Two new genera of operculate chytrids. *Amer. Jour. Bot.* 26:459-463.
- . 1941. A developmental study of three saprophytic chytrids. II. *Catenochytridium carolineanum* Berdan. *Amer. Jour. Bot.* 28:901-911.
- FOLPMERS, T. 1921. Der Zersetzung des Chitins und des Spaltungproductes desselben, des Glucosamins, durch Bakterien. *Chem. Wochenbl.* 18:249.
- HANSON, ANNE M. 1944. Three new saprophytic chytrids. *Torrey* 44:30-33.
- HOCK, C. W. 1940. Decomposition of chitin by marine bacteria. *Biol. Bull.* 79:199-206.
- INGOLD, C. T. 1944. Studies on British chytrids. II. A new chytrid on *Ceratium* and *Peridinium*. *Trans. Brit. Mycol. Soc.* 27:93-95.
- JOHNSON, DELIA A. 1932. Some observations on chitin-destroying bacteria. *Jour. Bact.* 24:335-340.
- KARLING, J. S. 1941. *Cylindrochytridium johnstonii*, gen. nov. et sp. nov., and *Nowakowskiella profusum* sp. nov. *Bull. Torrey Bot. Club* 68:381-387.
- . 1944. Brazilian chytrids. II. New species of *Rhizidium*. *Amer. Jour. Bot.* 31:254-261.
- PETERSEN, H. E. 1903. Note sur les Phycomycètes observés dans les téguments vides nymphes de Phryganes avec description de trois espèces nouvelles de Chytridinées. *Jour. de Bot.* 17:214-222.
- SPARROW, F. K. 1937. Some chytridiaceous inhabitants of submerged insect exuviae. *Proc. Amer. Philos. Soc.* 78:23-53.
- ZOBELL, C. E., AND S. C. RITTENBERG. 1938. The occurrence and characteristics of chitinoclastic bacteria in the sea. *Jour. Bact.* 35:275-287.

FURTHER STUDIES ON THE CHIASMATA OF THE ALLIUM CEPA X A. FISTULOSUM HYBRID AND ITS DERIVATIVES¹

S. L. Emsweller² and H. A. Jones³

IN ALL varieties of *Allium cepa* L. examined by the writers (1935a) the chiasmata have been arranged at random in each bivalent; by mid-metaphase they become terminal or sub-terminal, and either rod or ring bivalents are formed. This situation in *A. cepa* was also reported by Levan (1936) and by Maeda (1937). Cochran (1942) reported the same for *A. ascalonicum* L.

In *A. fistulosum* L. the typical metaphase bivalent is held together by two chiasmata, one on each side of the centromere. This localization of metaphase chiasmata results in cruciform bivalents. Localized chiasmata have not been reported in *A. cepa*. However, Levan (1933), Emsweller and Jones (1935a), and Maeda (1937) agree that in *A. fistulosum* some bivalents are occasionally held together by random chiasmata at first metaphase. The average of 2.01 chiasmata per bivalent reported by Cochran (1942) in *A. fistulosum* also indicates the occurrence of a few random bivalents. The frequency of randomized bivalents apparently varies in different varieties of *A. fistulosum*. Levan (1933) compared four forms, of which three averaged less than one random chiasma per cell, while the fourth had from one to two per cell. In *A. fistulosum* var. Hidanegi, Maeda found about 94 per cent of the bivalents to have localized chiasmata; the remainder falling into several distinct types with randomized chiasmata. It was not mentioned whether or not the same bivalent was always non-localized.

The behavior of the chiasmata at first metaphase in the hybrid between *A. fistulosum*, with localized chiasmata, and *A. cepa*, with randomized chiasmata, has been described by the authors (1935a), Levan (1936), and Maeda (1937). All are in agreement that only random chiasmata occur in the F₁ plants. Recently Cochran (1942) reported on a species hybrid between *A. ascalonicum*, with random chiasmata, and *A. fistulosum*. The cross was made reciprocally, and in all the F₁ plants examined the chiasmata were random.

The first hybrid we obtained was between an inbred line of *A. cepa* var. Yellow Globe Danvers and an inbred line of the Nebuka type of *A. fistulosum*. Levan's hybrid was between the variety Braunschweiger of *A. cepa* and a form of *A. fistulosum* from a European botanic garden. The cross made by Maeda was between *A. cepa* var. Yellow Danvers and *A. fistulosum* var. Hidanegi.

In respect to frequency of bivalent formation at first metaphase, Maeda's hybrid and ours were

similar, each showing about 70 per cent pairing. In Levan's hybrid the frequency of bivalent formation was only about two per cent. No polyvalents were found in Maeda's hybrid and only one, a trivalent, in ours. In Levan's hybrid there were 14 trivalents, four quadrivalents, and one hexavalent. Maeda examined 44 cells, Levan 100, and Emsweller and Jones 130.

Maeda obtained seeds from both the F₁ and backcrosses to *A. fistulosum*. Levan did not secure any seeds from the F₁ under controlled conditions and all his attempts at backcrossing proved unsuccessful. In our hybrid no selfed seed has ever been secured under controlled pollinations. Numerous backcrosses to both *A. cepa* and *A. fistulosum* have been successful, but only when the hybrid was the pollen parent. Cochran's hybrid also was highly self-sterile; only six F₂ seeds were secured from the *A. fistulosum* × *A. ascalonicum* cross and no F₂ seed from the reciprocal. His hybrid was slightly backcross-fertile to both parents, but only when used as the pollen parent.

In a preliminary note by Emsweller and Jones (1935b) on the first backcross to *A. fistulosum* it was reported that 10 of 17 plants had localized chiasmata while the remaining seven had randomized chiasmata. From these data it was considered that localization of chiasmata may be controlled by a recessive gene, although it was pointed out that the population was small. The detailed data on frequency of randomized and localized bivalents in the first backcross plants were not published at that time because it was planned to grow additional generations for further observation.

Shortly after our preliminary note appeared Maeda published his paper on the F₁, F₂, and backcross between *A. fistulosum* and *A. cepa* (1937). Cytological studies of 1,000 gemini of the *A. fistulosum* parent showed that 94 per cent had localized chiasmata, and 1,000 in the *A. cepa* parent all had randomized chiasmata. Of the nine F₁ plants secured from a cross between *A. fistulosum* var. Hidanegi and *A. cepa* var. Yellow Danvers, only one was studied closely since it was found by preliminary examination that no cytological differences existed between any of them. Thirteen F₂ plants were secured; nine of these had bivalents with randomized chiasmata only, two had six bivalents that were always randomized and two always localized, while the other two plants had a constant arrangement of five randomized and three localized. The bivalents with localized chiasmata were of definite sizes in every case and it was concluded that the same chromosome pair was always involved.

A backcross population of 21 plants was also secured by Maeda by pollinating a hybrid between the *A. fistulosum* varieties Wasenegi × Kujonegi

¹ Received for publication March 17, 1945.

² Principal horticulturist, and ³ principal olericulturist, both of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Beltsville, Maryland.

with the species hybrid (*A. fistulosum* var. Hidanegi) \times (*A. cepa* var. Yellow Danvers). Six of these 21 plants had random chiasmata only, three had $7R + 1L$, seven had $6R + 2L$, three had $5R + 3L$, and two had $4R + 4L$. Here, too, the localized bivalents were always the same size, indicating a definite pair in each instance.

Levan (1941) reported on the cytology of six F_2 plants from seed set on the hybrid when isolated at least 400 meters from the parent species. All six plants were polyploids: two were triploid with 24 chromosomes, three were hypotetraploids with 30 and 31 chromosomes, and one had 32 chromosomes. Four of these (the two triploid plants, one hypotetraploid with 31 chromosomes and another with 32) were grown to flowering. Detailed cytological investigations were made of one of the triploids and the tetraploid. In the triploid the frequency of trivalents was high with most of the chiasmata being localized, but both random and local were found in the same cell. From both pollen mother cells and root tips it was determined that this plant had one *A. cepa* and two *A. fistulosum* satellited chromosomes.

In the plant with 32 chromosomes, the most commonly occurring configuration was the typical *A. cepa* ring bivalent. No localized chiasmata were observed. A few trivalents and quadrivalents occurred, the frequency being less than one per cell. The chromosomes usually separated regularly with 16 to each pole, and about 50 per cent of the pollen appeared to be good. At first the plant was considered highly fertile since all capsules were observed to swell and seeds containing embryos were formed. Prior to dehiscence of the capsules, however, the seeds began to atrophy and practically all rotted. Only one seedling was finally secured.

Later Jones and Clarke (1942) described a spontaneous amphidiploid from a cross between the *A. cepa* var. Australian Brown and *A. fistulosum*. This amphidiploid was about 50 per cent as self-fertile as the *A. cepa* and *A. fistulosum* parents. They did not observe any quadrivalents, but found a high frequency of 16 bivalents in which both randomized and localized types occurred in a ratio of approximately 1:1, with a slight excess of random types. This differs considerably from Levan's amphidiploid type, which was highly sterile, with some quadrivalents, and with the chiasmata always randomized.

The *A. fistulosum* \times *A. ascalonicum* hybrid obtained by Cochran was backcrossed as pollen parent to both *A. fistulosum* and *A. ascalonicum*. All of the plants in the *A. ascalonicum* backcross population had random chiasmata. The 104 plants in the backcross to *A. fistulosum*, however, were of eight types: 42 had random chiasmata only, 47 had localized only, six had $7R + 1L$, two had $6R + 2L$, one had $4R + 4L$, one had $3R + 5L$, one had $2R + 6L$, and four had $1R + 7L$. In the six plants having seven randomized and one localized bivalent, the latter was always of a definite size and shape,

indicating that the same two chromosomes were always involved. In all other plants that had both random and local bivalents, the chromosome pairs were definitely of one type or the other.

MATERIALS AND METHODS.—First backcross plants to *A. fistulosum* were selfed to obtain further information on the frequency of random and local bivalents in their progenies. One of the six selfed plants had random chiasmata only, two had an occasional localized bivalent; the remaining three had predominantly localized pairing, with few random bivalents. In addition a second backcross to *A. fistulosum* was made using as the pollen parent a first backcross to an *A. fistulosum* plant with 98 per cent randomized chiasmata.

The first hybrid plants and backcrosses to *A. fistulosum* were grown outdoors at Davis, California. Later they were moved to the Plant Industry Station, Beltsville, Maryland, where they as well as all subsequent populations were grown in the greenhouse. All have behaved as perennials.

A number of hybrids involving *A. fistulosum* and several different varieties of *A. cepa* have been grown and examined. Many backcrosses to *A. cepa* have also been studied.

The various populations are designated as: the species hybrids, first backcross to *A. cepa*, first backcross to *A. fistulosum*, second backcross to *A. fistulosum*, and selfed backcrosses to *A. fistulosum*.

The present paper includes the observations on all these progenies with special reference to the types of chiasmata found.

All observations are from pollen-mother-cell smears stained in acetocarmine. The photomicrographs were made at a magnification of $\times 1,200$ and reduced to ca. $\times 900$ for reproduction.

OBSERVATIONS.—A typical diaphase in a pollen mother cell of *A. cepa* is shown in figure 1, A. The chiasmata are all arranged at random, and at mid-metaphase following terminalization only rings and rods are found. The Nebuka type of *A. fistulosum* used in the crosses was similar to Levan's forms 1, 2, and 4 in which random bivalents occurred with a frequency of less than one per cell. Figure 1, B, C, and D are all from the same plant of *A. fistulosum* and from the same slide. They show (B) complete localization, (C) $1R$ (a ring) + $7L$, and (D) $1R$ (a rod) + $7L$. Thus in the Nebuka type of *A. fistulosum* at least one bivalent may have either random or local chiasmata. This is also true of the *A. fistulosum* used by Levan (1936) and by Maeda (1937). The frequency of non-localized bivalents in our strain of *A. fistulosum* is considerably less than one per cell, and two have never been seen by us in the same cell. All the random bivalents seen were of either the ring or the rod type, but it was impossible to determine whether it was always the same chromosome pair.

The species hybrids.—Recently it has been possible to examine cytologically 17 additional hybrids between nine different varieties of *A. cepa*, all crossed with the same inbred line of *A. fistulosum*.

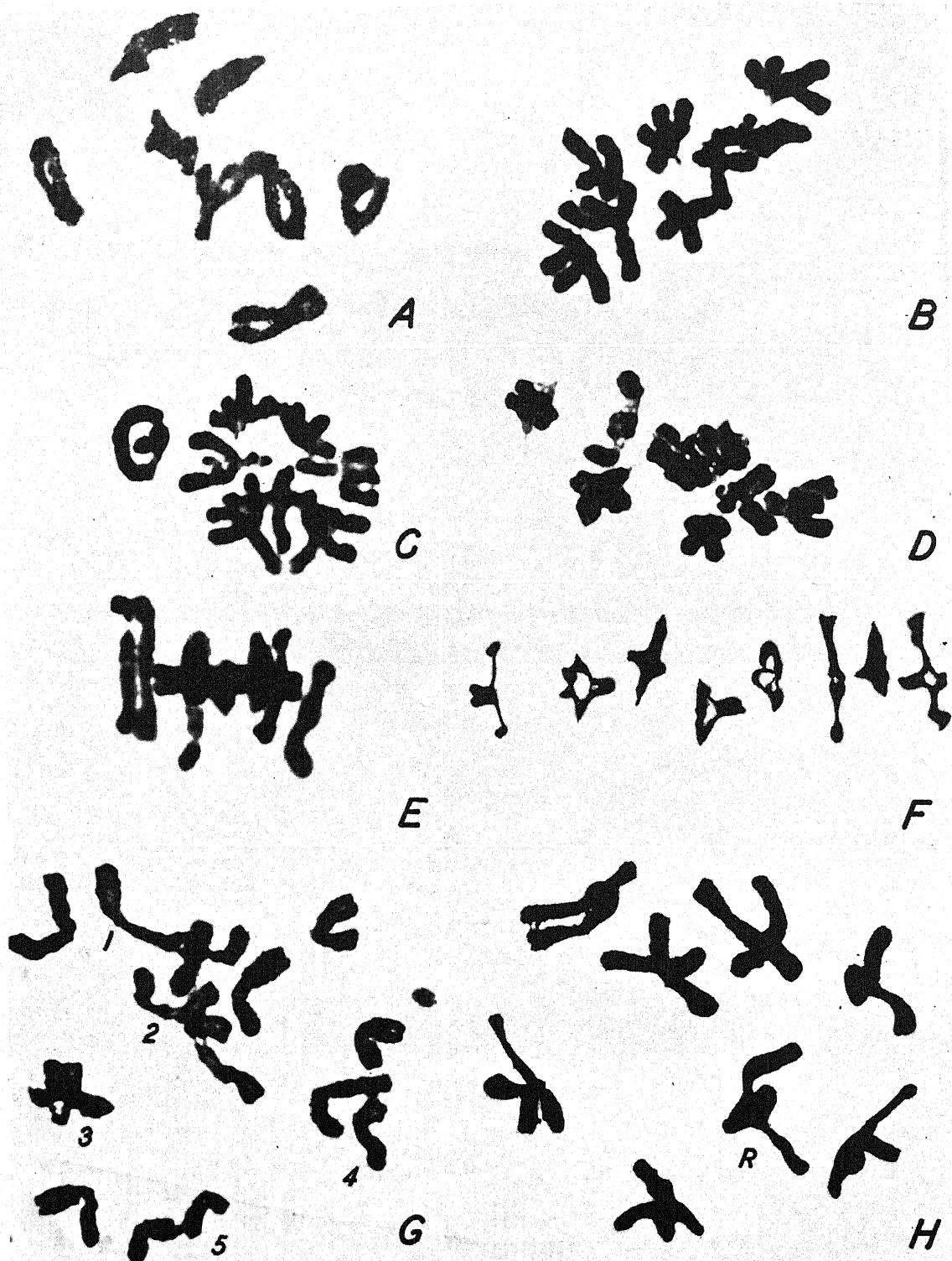


Fig. 1. A-H. Metaphase bivalents showing types of chiasmata in the hybrid *Allium cepa* \times *A. fistulosum* and its derivatives.—A. *A. cepa* \times 900.—B, C, D. *A. fistulosum*. \times 900.—E. Plant 17 of first backcross to *A. fistulosum*. \times 900.—F. *A. cepa* var. Yellow Globe Danvers \times *A. fistulosum* var. Nebuka. \times 900.—G. *A. cepa* var. Crystal Wax \times *A. fistulosum* var. Nebuka. \times 900.—H. Plant 10 of first backcross to *A. fistulosum*. \times 900.

TABLE 1. Chromosome pairing in 18 hybrids between 10 different varieties of *Allium cepa* and *A. fistulosum*.

Hybrids <i>Cepa</i> parent variety	Average number bivalents per p.m.c.	Type of pairing						
		$8_{II}+0_I$	$7_{II}+2_I$	$6_{II}+4_I$	$5_{II}+6_I$	$4_{II}+8_I$	$3_{II}+10_I$	$2_{II}+12_I$
Yellow Globe Danvers.....	7.59	94	24	9	1	2	0	0
Brigham Yellow Globe 1...	7.72	40	8	3	0	0	0	0
Brigham Yellow Globe 2...	7.72	41	11	2	0	0	0	0
Brigham Yellow Globe 3...	7.72	36	14	0	0	0	0	0
Brigham Yellow Globe 4...	7.62	31	19	0	0	0	0	0
Brigham Yellow Globe 5...	7.58	35	9	6	0	0	0	0
Brigham Yellow Globe 6...	7.51	30	20	1	1	0	0	0
Brigham Yellow Globe 7...	7.37	30	12	8	0	1	0	0
Brigham Yellow Globe 8...	7.36	22	24	2	1	0	0	0
Creole 1	7.27	24	19	6	2	0	0	0
Creole 2	4.28	0	1	3	15	8	9	2
White Portugal	7.86	43	7	0	0	0	0	0
California Early Red.....	7.36	20	21	2	1	0	0	0
Lord Howe Island.....	7.29	20	27	3	1	0	0	0
Australian Brown	7.24	32	28	12	1	0	0	0
Yellow Bermuda	7.24	27	24	10	1	0	0	0
Mountain Danvers	7.09	23	28	12	1	1	0	0
Crystal Wax	5.87	4	17	18	18	3	3	0
Maeda's hybrid	7.68	32	10	2	0	0	0	0
<i>A. fistulosum</i>								
<i>A. cepa</i>								
Levan's hybrid	5.98	8	26	19	18	6	3	1
<i>A. cepa</i>								
<i>A. fistulosum</i>								

The average number of chromosome pairs per pollen mother cell in these hybrids and in our first hybrid (with Yellow Globe Danvers) is shown in table 1. There are also included data from Maeda and Levan. Only those cells were counted that lacked fragments and multivalents.

Eight of our hybrids were between the Brigham Yellow Globe variety of *A. cepa* and *A. fistulosum*. While some variability in frequency of bivalent formation occurred in these eight plants, it is doubtful if it has any significance. The plants were grown in adjacent pots in the same greenhouse bench, and samples of pollen mother cells were collected from all at the same time. The difference in frequency of bivalent formation in our two hybrids between the *A. cepa* variety Creole and *A. fistulosum* was rather great and may have some significance, but

the numbers involved are too few to ascertain this point with any degree of reliability. The first of the two hybrids was made with a commercial Creole bulb as maternal parent while in the second the Creole bulb was from an inbred line. The hybrid between the *A. cepa* variety White Portugal and *A. fistulosum* had the highest frequency of bivalent formation. There was probably no significant difference in amount of pairing between the five hybrids involving the *A. cepa* varieties California Early Red, Australian Brown, Yellow Bermuda, Lord Howe Island, and Mountain Danvers. The one with Crystal Wax, however, was almost as irregular in chromosome pairing as the second Creole hybrid. None of the 18 hybrids produced any selfed seed.

If chromosome pairing is to be accepted as a

TABLE 2. Chromosome pairing in six first backcrosses to *A. cepa* plants.

	Average num- ber bivalents per p.m.c.	Type of pairing		
		$8_{II}+0_I$	$7_{II}+2_I$	$6_{II}+4_I$
Brigham Yellow Globe (Yellow Bermuda \times <i>A. fistulosum</i>).....	7.9	45	1	0
Australian Brown (<i>A. cepa</i> \times <i>A. fistulosum</i>).....	7.9	53	3	0
Early Yellow Globe (Ebenezer \times <i>A. fistulosum</i>)...	7.9	49	4	0
Early Yellow Globe (Brigham Yellow Globe \times <i>A. fistulosum</i>)	7.9	45	5	0
Australian Brown (Australian Brown \times <i>A. fistulosum</i>)	7.8	42	9	0
Early Yellow Globe (Australian Brown \times <i>A. fistulosum</i>)	7.5	30	19	3

criterion of relationship, the data in table 1 indicate that some varieties of *A. cepa* may be much closer to *A. fistulosum* than others. These differences in relationship are also indicated by the cytological evidence of different types of bivalent configurations found in the hybrids. For instance, in figure 1, F and G are shown first metaphases from two different hybrids. The first is from our initial hybrid between *A. cepa* var. Yellow Globe Danvers and *A. fistulosum*. The eight bivalents are closely paired,

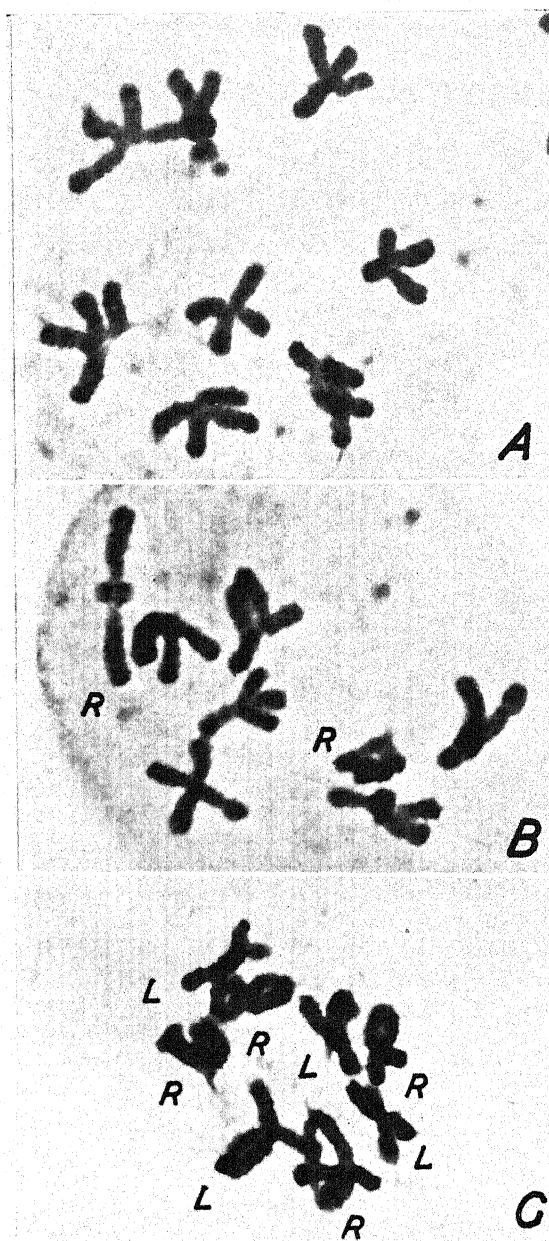


Fig. 2. A-C. Metaphase bivalents showing types of chiasmata in second backcross to *A. fistulosum*.—A. Plant 5 with all chiasmata localized. $\times 900$.—B, C. Plant 18, B with 2 randomized and 6 localized bivalents, C with 4 randomized and 4 localized. $\times 900$.

five being rings and three rods. They are very similar to those found in *A. cepa*. In figure 1, G, from the hybrid *A. cepa* var. Crystal Wax \times *A. fistulosum*, there are only three closely paired bivalents (1, 2, 3), two very loose associations (4, 5), six univalents, and a fragment. In those hybrids in which a low frequency of bivalent formation occurred the chromosomes were paired mostly by loose terminal associations. In those with a high frequency of pairing, typical *A. cepa* rings and rods predominated. The latter plants also had higher chiasma frequencies. Levan's hybrid is apparently very similar to our Crystal Wax \times *A. fistulosum*, or Creole No. 2 \times *A. fistulosum*, whereas Maeda's more nearly approximates the one with White Portugal, Yellow Globe Danvers, or one of those in which Brigham Yellow Globe was the *A. cepa* parent. Since the same inbred line of *A. fistulosum* was used in all our hybrids, the variability in frequency and type of bivalents formed indicates the existence of considerable variability within the *A. cepa* varieties.

First backcross to A. cepa.—Many plants of the first backcross to *A. cepa* have been examined cytologically and no bivalents with localized chiasmata were observed. The frequency of bivalent formation in six of these plants selected at random from the available populations is presented in table 2. In each instance a different F_1 plant was used as the pollen parent. The plants, as expected, had a higher frequency of chromosome pairing than was found in the F_1 individuals. The bivalents were all typical of *A. cepa*, and loose associations, such as occurred in some F_1 plants, were rare. Two plants from backcrosses to *A. cepa*, Brigham Yellow Globe (Yellow Bermuda \times *A. fistulosum*) and Australian Brown (*A. cepa \times *A. fistulosum*), were as regular in chromosome pairing as *A. cepa* var. Yellow Globe Danvers. In spite of the high regularity of chromosome pairing found in some of these plants they have been, on the whole, highly self-sterile. Some individuals when self-pollinated during the past few years have yielded small amounts of seed, but most of them have produced no seed. Some second and third backcrosses to *A. cepa* have been made, but no data are available on the type of chiasmata or fertility of these individuals.*

First backcross to A. fistulosum.—The data from the 17 plants secured by backcrossing the F_1 plant to *A. fistulosum* are presented in table 3. The data on chromosome pairing and types of chiasmata were taken from different slides, hence the number of cells included in one group of data may not agree with the other. The plants are arranged in the order of percentage of localization of their chiasmata. Plants 1 and 2 had all bivalents localized and plant 17 had none localized. The other plants had both randomized and localized bivalents in the same cell, but none were as regular in this respect as the first backcross plants described by Maeda (1937). Ten of the 17 plants had bivalents predominantly localized. Even plants 9 and 10, which averaged 0.75

TABLE 3. Chromosome pairing, percentage of localization, and fertility in first backcross to *A. fistulosum*.

Plant number	Number cells showing chromosome pairing as indicated			Average number bivalents per p.m.c.	Frequency of cells with randomized and localized bivalents as indicated							Per cent localized bivalents	Number seed per umbel
	8 _{II} +0 _I	7 _{II} +2 _I	6 _{II} +4 _I		8L+0R	7L+1R	6L+2R	5L+3R	2L+6R	1L+7R	0L+8R		
1	64	0	0	8.0	8	0	0	0	0	0	0	100	292.0
2	52	1	0	7.9	8	0	0	0	0	0	0	100	66.0
3	50	0	0	8.0	9	1	0	0	0	0	0	99	122.0
4	56	0	0	8.0	6	1	0	0	0	0	0	98	231.0
5	51	2	0	7.9	6	3	0	0	0	0	0	96	22.0
6	56	0	0	7.9	7	1	1	0	0	0	0	96	13.0
7	58	1	0	7.9	5	2	1	0	0	0	0	94	51.0
8	91	2	1	7.9	7	0	2	1	0	0	0	91	88.0
9	55	1	0	7.9	4	2	2	0	0	0	0	91	114.0
10	85	5	0	7.9	6	3	3	0	0	0	0	91	205.0
11	50	0	0	8.0	0	0	0	0	1	8	0	14	1.0
12	40	20	2	7.6	0	0	0	0	2	4	3	11	6.0
13	52	20	3	7.6	0	0	0	0	0	6	3	8	.1
14	41	8	3	7.7	0	0	0	0	0	1	6	2	8.0
15	54	1	0	7.9	0	0	0	0	0	1	7	2	6.0
16	45	3	1	7.9	0	0	0	0	0	1	9	1	.2
17	44	10	0	7.8	0	0	0	0	0	0	9	0	6.0

non-localized bivalents per cell, were well within the frequency of 1.67 per cell reported by Levan as normal for his No. 3 strain of *A. fistulosum*. Plants 11 to 17 had predominantly randomized chiasmata. Here, too, there was not a constant arrangement of 7R + 1L or any other ratio. Plant 17 was identical with *A. cepa* as far as non-localization was concerned. It, however, had more univalents than we have ever seen in any *A. cepa*. Figure 1, H is a cell from plant 10 with one random and seven local bivalents. Figure 1, E is from plant 17 with all chiasmata random.

The fertility of these plants, as expressed in number of seed per umbel, is also shown in table 3.

All flower heads on each plant were self-pollinated and, as noted, there was a considerable difference in seed setting between the 10 plants with predominantly localized chiasmata and the seven with mostly randomized chiasmata. Plants with localized chiasmata averaged 120 seeds per head and those with randomized only 3.9. This difference could scarcely be attributed to decreased chromosome pairing, which averaged 98 per cent for the 10 fertile plants and 93 per cent for the seven with low fertility.

Second backcross to A. fistulosum.—The second backcross to *A. fistulosum* was made by using pollen of plant 14 from the first backcross to *A. fistu-*

TABLE 4. Chromosome pairing and frequency of randomized and localized bivalents in second backcross to *A. fistulosum*.

Plant number	Number cells showing chromosome pairing as indicated			Average number bivalents per p.m.c.	Frequency of cells with randomized and localized bivalents as indicated						Per cent localized bivalents
	8 _{II} +0 _I	7 _{II} +2 _I	6 _{II} +4 _I		8L+0R	7L+1R	6L+2R	5L+3R	4L+4R	3L+5R	
1	25	0	0	8.0	24	1	0	0	0	0	99
2	23	1	0	7.9	18	6	0	0	0	0	97
3	25	0	0	8.0	20	5	0	0	0	0	97
4	25	0	0	8.0	16	9	0	0	0	0	95
5	40	0	0	8.0	23	15	2	0	0	0	94
6	32	0	0	8.0	18	13	1	0	0	0	94
7	31	0	0	8.0	16	14	1	0	0	0	93
8	24	1	0	7.9	12	10	3	0	0	0	92
9	26	0	0	8.0	10	14	2	0	0	0	91
10	19	9	0	7.6	0	24	3	1	0	0	89
11	22	5	0	7.8	5	19	3	0	0	0	88
12	10	9	0	7.5	0	18	0	1	0	0	86
13	27	3	0	7.9	0	20	10	0	0	0	83
14	15	1	0	7.9	0	8	7	1	0	0	80
15	7	18	0	7.2	0	7	16	1	1	0	77
16	12	12	1	7.4	0	0	4	9	9	3	60
17	29	0	0	8.0	0	0	0	18	11	0	58
18	12	0	0	8.0	0	0	1	4	4	3	53

[illegible]

and Jones (1935a and b), Maeda (1937), Levan (1936, 1941), and Jones and Clarke (1942), on localization of chiasmata in F_2 and backcross progenies of the species hybrid *A. cepa* \times *A. fistulosum* appear contradictory. The data presented by Cochran (1942) from the hybrid *A. fistulosum* \times *A. ascalonicum* may also be included here, if we assume that *A. ascalonicum* is analogous to *A. cepa* in the hybrid with *A. fistulosum*.

There are some points upon which all the above investigators agree. For instance, all have reported only random chiasmata in *A. cepa* and occasional non-localized bivalents in *A. fistulosum*. Cochran (1942) also reports only random bivalents in *A. ascalonicum*. In the F_1 plants so far examined, only random chiasmata have been observed. In the F_2 and the backcross to *A. fistulosum*, individual plants have been found in which both local and random bivalents occurred in the same cell. This is essentially, as pointed out earlier, the normal condition for *A. fistulosum*.

The differences reported are concerned with percentage of complete pairing observed, fertility of the hybrids, frequency of randomized and localized bivalents in plants of backcross progenies, and the uniformity of types of bivalents in an individual plant. In addition there has been a considerable difference in the number of plants observed which could be classified as all randomized or all localized or of some intermediate type having both kinds of bivalents present.

Allium cepa has been in cultivation for a long time and the wild form is unknown. The varieties used in producing these hybrids vary considerably from one another in many characters. As may be inferred from their names, they originated in widely separated regions where they were probably selected because of favorable reaction to a particular environment.

There are also a large number of horticultural varieties of *A. fistulosum*. Levan (1933) mentions some 50 forms secured from various botanical gardens, and reports uniformity of cytological characters in somatic chromosomes, but a few minor differences in meiosis. Felix (1933), in his note on disease resistance in *A. fistulosum*, lists four varieties of the Japanese onion Nebuka, namely, Natsunegi, Sinju-negi, Tokyo, and Itwatsuki. Maeda (1937), in his study of the species hybrid and its derivatives, used three garden varieties of *A. fistulosum*. These were Hidanegi, Wasenegi, and Kujonegi. Probably the differences in chromosome pairing and fertility of the various hybrids reflect the genic variability within both *A. cepa* and *A. fistulosum*.

As already pointed out, in the first backcross to *A. fistulosum* secured by us the 17 plants were classified into two distinct groups, one with random and the other with local chiasmata. Most of the plants had both randomized and localized bivalents in the same cell but, unlike Maeda's (1937) first backcross population, none had a constant ratio of

so many random to so many local. There were two plants with localized chiasmata only and eight others in which most of the bivalents were of this type. Maeda's backcross population of 21 plants had very few localized bivalents and no plants with this type of chiasmata only. In his plants with both sorts present, the ratios were consistently 7R to 1L, 6R to 2L, 5R to 3L, or 4R to 4L. In Cochran's backcross to *A. fistulosum*, mentioned earlier, he also found plants with both types of chiasmata present in the same cell, and always in the same ratio. Unlike Maeda (1937), however, 42 of the 104 plants had random bivalents only and 47 localized only. The four plants with 1R + 7L, and the one plant with 2R + 6L could have been classified with the 47 localized plants since *A. fistulosum* does have occasional random bivalents. If only 50 or fewer plants had been examined the chances are that random and local individuals only would have been found.

Excluding Maeda's data, the evidence from first backcrosses indicates a genic explanation for inheritance of localized chiasmata. The data, however, from our second backcross to *A. fistulosum* and our progenies from selfing first backcross to *A. fistulosum* plants do not support this hypothesis. The second backcross to *A. fistulosum* was made with first-backcross plant 14 as pollen parent, and the same plant was also self-pollinated. As pointed out earlier, there were no plants from the second backcross to *A. fistulosum* that had random chiasmata only. The population differed considerably from our first backcross to *A. fistulosum*. The 18 plants secured could not be separated into two distinct classes on the basis of the type of chiasmata present. Most of the plants had a high frequency of localized bivalents, and no plant had more than five with random chiasmata. In plants 16 and 18 there were at least three bivalents that had chiasmata random in some cells and localized in others, and in all other plants there were either one or two bivalents in this category.

The 24 plants in the population, secured by selfing plant 14 from the first backcross to *A. fistulosum*, differ considerably from the second backcross to *A. fistulosum* plants and also from Maeda's F_2 (1937). Nine of these 24 plants had random chiasmata only, four others averaged 99 per cent randomized, and in seven others over 90 per cent of the bivalents had random chiasmata. In the remaining four plants the bivalents were mostly localized but none had all bivalents of this type. While there is some superficial indication of a 3:1 segregation in this population, the data do not justify this assumption.

In the amphidiploid of Jones and Clarke, the 32 chromosomes paired as bivalents. In some cells it was possible to distinguish eight pairs with randomized and eight with localized chiasmata, but frequently there was an excess of random types. It was suggested that while the pairing was mostly *A. cepa* with *A. cepa* and *A. fistulosum* with *A. fistu-*

losum, there might also be some pairing of *A. cepa* with *A. fistulosum*. It should also again be pointed out that at least one bivalent is often random in *A. fistulosum* itself.

The data presented by Maeda indicated that only definite chromosome pairs form cruciform configurations. From this it may be inferred that localization of chiasmata occurs only when two *A. fistulosum* chromosomes pair. If this were the situation and random assortment of chromosomes took place in the F_1 , it would be difficult to understand the F_2 population secured by Maeda and also the results of selfing our first backcross plant that had random chiasmata. According to theoretical expectation the various types of plants should have occurred with the following frequency: $8R + 0L$, 11 per cent; $7R + 1L$, 13 per cent; $6R + 2L$, 18 per cent; $5R + 3L$, 21 per cent; $4R + 4L$, 19 per cent; $3R + 5L$, 11 per cent; $2R + 6L$, 4 per cent; $1R + 7L$, .73 per cent, and $0R + 8L$, .04 per cent. Actually Maeda secured nine plants (69+ per cent) with all chiasmata random, two (15+ per cent) with $6R + 2L$, and two (15+ per cent) with $5R + 3L$. In our population there were nine all random plants, and 15 that could not be classified because of the variable numbers of random and local bivalents within each plant.

The theoretical distribution of plants in Maeda's backcross to *A. fistulosum* would be as follows: $8R + 0L$, .39 per cent; $7R + 1L$, 3+ per cent; $6R + 2L$, 11+ per cent; $5R + 3L$, 22+ per cent; $4R + 4L$, 27+ per cent; $3R + 5L$, 22+ per cent; $2R + 6L$, 11+ per cent; $1R + 7L$, 3+ per cent, and $0R + 8L$, .39 per cent. He actually found 6 plants with $8R + 0L$ (28+ per cent), 3 with $7R + 1L$ (14+ per cent), 7 with $6R + 2L$ (33+ per cent), 3 with $5R + 3L$ (14+ per cent), and 2 with $4R + 4L$ (9+ per cent).

The population of 104 plants observed by Cochran (1942) should have shown about .39 plants all random, 3 plants $7R + 1L$, 11 plants $6R + 2L$, 22 plants $5R + 3L$, 28 plants $4R + 4L$, 22 plants $3R + 5L$, 11 plants $2R + 6L$, 3 plants $1R + 7L$ and .39 plants all L . The actual distribution was 42-6-2-0-1-1-1-4-47.

The differences between Levan's (1941) tetraploid and the one reported by Jones and Clarke (1942) indicate a different type of origin for each. In Levan's plant localized chiasmata were not found while in the other as many as eight localized bivalents per cell were not uncommon. Very probably the latter was a true amphidiploid arising from the doubling of the chromosome number of an F_1 zygote. The 32 chromosomes would then be 16 *A. fistulosum* and 16 *A. cepa*, both unmodified. In Levan's plant the random arrangement of all chiasmata indicates that possibly two post-meiotic unreduced gametes functioned, or the plant was some sort of autotetraploid derived from spontaneous chromosome doubling in a zygote formed by union of two gametes composed predominantly of *A. cepa* chromosomes. The high failure of chromosome pair-

ing in Levan's hybrid tends to support this assumption since most of the post-meiotic chromosomes were probably unmodified by crossing over. However, the frequency of less than one polyvalent per cell reported by Levan (1941), as compared with 6.5 per cell reported by Toole and Clarke (1944) in their colchicine-induced *A. cepa* autotetraploid, indicates that Levan's plant was probably not a simple tetraploid of *A. cepa*.

The results of our first backcross to *A. fistulosum* indicated a gene mechanism for control of localization of chiasmata. In the light of other data, however, it now appears more likely that the 1:1 ratio of random and local plants is the result of the elimination of practically all gametes other than those with chromosomes predominantly *A. fistulosum* or *A. cepa*. This explanation was first suggested to us by Philip C. Burrell and was also offered by Cochran to explain his backcross population. Since approximately equal numbers of each type of gamete would be formed, the 1:1 ratio would naturally result. The gametes, however, are probably mostly composed of modified *A. cepa* and *A. fistulosum* chromosomes. From the data in table 1 it can be seen that a considerable amount of chromosome pairing occurs in some hybrids. As a result, decidedly different types of gametes would be formed. In some instances the post-meiotic chromosomes would be composed of both *A. cepa* and *A. fistulosum* segments in varying proportions. This alone would account for some of the variability reported in backcross populations.

As pointed out earlier, the constancy of the ratio of random to local bivalents in the backcross plants of Maeda and Cochran was not found in any of our plants. The two chiasmata of a localized bivalent are always one on each side of the centromere. This suggests that some feature of the centromere region of *A. fistulosum* chromosomes differs from the same sector in *A. cepa* chromosomes. Probably then, localization of chiasmata can only occur when the members of a bivalent are homologous for this segment of *A. fistulosum*. In the hybrids it is probable that little crossing over occurs in the centromere region. As a result no localized chiasmata are formed. The post-meiotic chromosomes which function in making the backcross populations are probably composed of both *A. cepa* and *A. fistulosum* sectors. This would be especially true in those instances where the hybrid had a high frequency of chromosome pairing. Thus backcross plants involving different hybrids with *A. fistulosum* could be expected to give varying results as to types of chiasmata in their bivalents. In the new chromosomes with *A. fistulosum* centromere regions and *A. cepa* sectors in the two arms, the chiasmata would likely be localized in some instances and randomized in others depending on where they were formed. As backcrossing to *A. fistulosum* was continued the percentage of localized bivalents in the entire population would be expected to rise. That this occurred may be seen by comparing tables 3 and 4. In the

first backcross 59 per cent of the bivalents had localized chiasmata, in the second backcross 84 per cent of the bivalents had localized chiasmata. This increase occurred even though first-backcross plant 14 used to produce a second backcross had almost 100 per cent randomized bivalents. If any of the first 10 plants of table 3 had been used to produce the second backcross it is probable that the entire population would have had very few randomized chiasmata. Likewise the data from selfing plants of the first backcross to *A. fistulosum* show that those individuals with a high frequency of localized bivalents produced plants with a similar condition, whereas those with a high frequency of randomized pairing gave progenies with mostly random bivalents. The apparent 3:1 ratio for random versus localized pairing in the progeny of plant 14 (table 5) is what would be expected if only gametes predominantly like *A. cepa* or *A. fistulosum* functioned. Probably many of the chromosomes of plant 14 that produced this population were of *A. cepa* origin, hence the majority of the 24 plants in its selfed progeny had random bivalents.

The much greater fertility of the first-backcross plants with localized chiasmata (table 3) is rather interesting. The ten fertile plants averaged 7.93 bivalents per cell, and the seven others had 7.78. This slight variation is probably not significant and cannot account for the great difference in fertility. All the more fertile plants had localized chiasmata and probably had fewer segments of *A. cepa* chromosomes, so were predominantly of the *A. fistulosum* genotype. The sterile plants, on the other hand, probably had more blocks of *A. cepa* genes in their chromosomes since their chiasmata were mostly randomized. This was indicated rather clearly by the appearance of the two groups of plants. The fertile individuals were almost identical with the *A. fistulosum* parent, while the sterile plants all had some characteristics of *A. cepa*.

SUMMARY

In *A. fistulosum* the chiasmata of metaphase bivalents are located one on each side of the centromere.

The result is a cruciform configuration differing considerably from the rings and rods found in *A. cepa*, in which the chiasmata are all randomized. In hybrids between these species the chiasmata are all arranged at random as in *A. cepa*. Investigations of these hybrids have resulted in conflicting reports concerning the behavior of the chiasmata in plants from the backcross to *A. fistulosum*. These differences are concerned chiefly with fertility, frequency of both randomized and localized bivalents, constancy of the number of randomized and localized bivalents in a plant, and the frequency of highly randomized and localized plants.

The 1:1 ratio of randomized to localized plants in our backcross population is believed to be the result of elimination of most gametes not predominantly *A. fistulosum* or *A. cepa*. Thus, backcrossing to *A. fistulosum* results in about one-half the plants having randomized and the other half localized bivalents.

In many of the plants from the backcross to *A. fistulosum* some bivalents may have either randomized or localized chiasmata. This probably results from pairing between an *A. fistulosum* chromosome and a post-meiotic chromosome from the hybrid. The latter in some instances will contain segments from both *A. cepa* and *A. fistulosum*. These new chromosomes should be almost pure *A. cepa* or *A. fistulosum* for a short distance on each side of the centromere. If a bivalent in a backcross plant forms chiasmata on both sides of, and close to the centromere, it may be localized provided other chiasmata are not formed in either arm. If chiasmata are formed in the arms of the chromosomes only, the same bivalent may be randomized.

First backcross plants that have predominantly localized bivalents give progeny with a similar type of chiasmata. This is also true for plants with randomized chiasmata.

The greater fertility of first-backcross plants with localized chiasmata is to be expected since these plants more closely approach the *A. fistulosum* genotype.

BUREAU OF PLANT INDUSTRY STATION,
BELTSVILLE, MARYLAND

LITERATURE CITED

- COCHRAN, F. D. 1942. Cytogenetic studies on the species hybrid *Allium fistulosum* and *Allium ascalonicum* and its backcross progenies. Thesis Univ. of Calif. Library.
- EMSWELLER, S. L., AND H. A. JONES. 1935a. Meiosis in *Allium fistulosum*, *Allium cepa*, and their hybrid. *Hilgardia* 9: 277-294.
- , AND —. 1935b. A gene for control of interstitial localization of chiasmata in *Allium fistulosum*. *Science* 81: 543-544.
- FELIX, E. I. 1933. Disease resistance in *Allium fistulosum*. *Phytopath.* 23: 109-110.
- JONES, H. A., AND A. E. CLARKE. 1942. A natural amphidiploid from an onion species hybrid, *Allium cepa* × *Allium fistulosum*. *Jour. Hered.* 33: 25-32.
- LEVAN, A. 1933. Cytological studies in *Allium*. IV. *Allium fistulosum*. *Svensk. Bot. Tids.* 27: 211-232.
- . 1936. Die Zytologie von *Allium cepa* × *fistulosum*. *Hereditas* 21: 195-214.
- . 1941. The cytology of the species hybrid *Allium cepa* × *fistulosum* and its polyploid derivatives. *Hereditas* 27: 253-272.
- MAEDA, T. 1937. Chiasma studies in *Allium fistulosum*, *Allium cepa*, and their F₁, F₂ and backcross hybrids. *Jap. Jour. Genetics* 13: 146-159.
- TOOLE, M. G., AND A. E. CLARKE. 1945. Chromosome behavior and fertility of colchicine-induced tetraploids in *Allium cepa* and *A. fistulosum*. *Herbertia* (in press).

GROWTH AND VASCULAR DEVELOPMENT IN THE SHOOT APEX OF *SEQUIA* *SEMPERVIRENS* (LAMB.) ENDL. II. VASCULAR DEVELOPMENT IN RELATION TO PHYLLOTAXIS¹

Clarence Sterling

IN AN earlier paper (Sterling, 1945), the general cytohistology and organization of the shoot apex of *Sequoia sempervirens* were discussed. The present article in this series is concerned with phyllotaxis and the topography of vascularization in the shoot apex. Materials and techniques are identical with those described in the paper cited above. Drawings were made by the *camera lucida* technique and by microprojection. The methods of Esau (1942) and Crafts (1943a, b) were followed in the interpretation of serial longisections and transections.

It is the writer's opinion that an analysis of the course of the vascular bundles in relation to leaf arrangement will provide a background for the histological details of procambial development. In addition, such an analysis is useful in considering the more general morphological relationship between "leaf" and "stem" in the shoot. The reader is referred to Arber (1930, 1941), Schüpp (1936), and Eames (1936, pp. 380-386) for general discussions on the origin and construction of the leafy shoot.

RÉSUMÉ OF THEORIES DEALING WITH PHYLLOTAXIS.—Although several excellent histories and critiques of theories of leaf arrangement are to be found in the literature (Church, 1920; Snow and Snow, 1931; Hirmer, 1922), it seems necessary to sum up these theories in order to introduce one which is currently finding increasing factual support.

The successive leaves of a shoot are usually arranged in an ascending spiral or in alternating whorls. These leaves are spaced at relatively constant angular distances in the horizontal plane. The angular distances, known as "divergences," have also been expressed as fractions of the periphery of the shoot. At an early date, it was evident that certain ratios were very common: $1/2$, $1/3$, $2/5$, $3/8$, $5/13$, . . . in an apparent summation series.

This particular series, initiated by the fractions $1/2$ and $1/3$, was given the name of the mathematician, Fibonacci. Restricted between the values of $1/2$ (180°) and $1/3$ (120°), the fractional values of the series gradually approach the limiting angle of $137^\circ 30' 28.936''$. This angle then became the "ideal angle" for a certain school of botanists. Besides this primary series, there were also recognized various secondary series, or "Nebenkette," formed from these basic fractions:

1. $1/3$ and $1/4$: $2/7$, $3/11$, $5/18$. . . to a limiting value of $99^\circ 30' 6''$,
2. $1/4$ and $1/5$: $2/9$, $3/14$, $5/23$. . . to a limiting value of $77^\circ 57' 19''$, etc.

These latter series, however, occur more rarely than the Fibonacci fractions. (See Hirmer, 1922).

The theories advanced to explain the regularity

of leaf arrangement express, according to Snow and Snow (1931), two main views. These are as follows:

One view is based on the concept of the so-called "genetic spiral" (basic type of spiral formed by the successively produced leaves). Many theories have been formulated to account for the location of leaf primordia at a fairly constant divergence angle of $137\frac{1}{2}^\circ$. One theory relates arrangement of the leaf primordia to the securing of maximum illumination (Wiesner, 1907). Hirmer (1922) and Barthelmess (1935) have supported the more recent idea of the most equitable spatial arrangement ("die günstige Raumausnutzung") at the growing apex. Church (1920) has developed the concept of radiating growth impulses from the center of the shoot apex to explain the genetic spiral. This genetic spiral, according to all the above theories, is supposedly a result of inherent shoot relationships.

The second view envisages the basic spiral as a secondary phenomenon resulting from the influence of the so-called "contact parastichies." (In the $3/8$ phyllotactic system, a leaf primordium will be in contact with the 3rd and 5th older leaves at the apex when it arises. For leaf primordium "5," the series of parastichies would then be 8-11-14 . . . and 10-15-20 . . . See figure 1 at 160μ below the summit of the apical cone.) This theory, founded by the physiological anatomist Schwendener, states that the position of new primordia at the apex is determined by the physical "contact pressures" of older primordia—more specifically, of the primordia of the contact parastichies (explained briefly by Weisse, 1900).

GENERAL ASPECTS OF VASCULARIZATION IN THE SHOOT OF *SEQUIA*.—The vascular strands of the apex develop in the eumeristem tissue which remains after the pith mother cells differentiate. There is no procambial ring in the sense of Kostytschew (1922, 1924). Instead, the vascular tissue differentiates from the eumeristem in the form of individual strands, before any trace of cortical vacuolation is noticeable. At the level of insertion of the second or third primordium, the strands are arranged to form a cylinder. The bundles are separated from one another by undifferentiated eumeristem. Kaplan (1937) has characterized the latter as "Restmeristem" or residual meristem, which is not microscopically distinguishable from the neighboring tissue of the future cortical region at this stage.

There are no "cauline" bundles in the *Sequoia* shoot as distinct from bundles common to both stem and leaf. Each leaf has a single trace. Apart from these traces and those of the axillary buds, there are no other bundles in the redwood shoot. Even the traces to the buds could logically be called "leaf traces." (See below.)

¹ Received for publication March 12, 1945.

A study of successive transections and longisections has established the fact that procambial strands develop acropetally from the stem into the leaves. These strands are continuous with older ones from which they branch. They are present in the shoot before the leaves, into which they will run, arise at the shoot apex (fig. 1). See also figure 3 (Sterling, 1945) for a longisection indicating early acropetal procambial differentiation.

The apex is provided with a "rosette" of young leaf primordia in addition to several developing foliar buttresses. These primordia and buttresses already possess procambial strands which are continuous down to their points of branching from older traces. In addition, at various heights and in differing degree of development in the shoot below, can be found procambial strands, whose leaf primordia have little or no visible representation at the apical cone (traces "-2" to "-6" in fig. 1). The procambial strand thus appears in the vascular cylinder before its associated leaf primordium is recognizable either as a series of periclinal or as a hump on the flank of the apical cone (traces "-3" to "-6" in fig. 1). Also appearing to substantiate the claim of continuous, acropetal differentiation of the procambial strands is the fact that the farther the procambial cells of a strand are removed from the apex of the shoot, the greater is the extent of their differentiation. This last point will be discussed in greater detail in the third paper of the present series.

The evidence furnished by *Sequoia* and brought forth by Lestiboudois (1840), Priestley, Scott, and Gillett (1935), Smith (1941), Esau (1942), and Crafts (1943a) suggests that a third interpretation of phyllotaxy is possible for tracheophytes. According to this view, the arrangement of foliar members at the shoot apex is determined not solely by the mutual relationships of leaves and shoot apex but, in some measure, by the vascular structure of the shoot. The acropetal differentiation of foliar bundles in the shoot prior to any sign of emergence of the related leaf primordium can be considered a partial determinant of the position of that primordium among its fellow members in the leaf rosette.

LEAF AND LEAF TRACE ARRANGEMENTS IN SEQUOIA. —Cross-sectional series of about 25 terminal buds were studied. In this limited sample, five different types of phyllotaxy were observed, four of which consisted of two pairs of sinistrorse (cathodal) and dextrorse (anodal)² complements of each other. The fifth type is a sinistrorse spiral, whose dextrorse complement was not observed in this group but whose existence is rendered possible by the presence of the other pairs.

A few types of leaf arrangement are shown in figure 1 (at 160 μ) and figure 2 by means of diagrammatic transections. The reason for the *circa* preceding the phyllotactic fractions in figure 2 can be understood if one follows the lines drawn from

² A dextrorse spiral is one in which the course of the spiral rises from left to right on the surface towards the observer; the sinistrorse spiral rises from right to left.

the center of the shoot through the bundles of leaves of the supposed orthostichies in each diagram. That is, in a $3/8$ series in a strict sense, there should be eight vertical rows of leaves or orthostichies along the shoot. Hence, in cross section, a line drawn from the center of the shoot through these leaves should be straight. Actually such a line curves very definitely. Thus there is no vertical orthostichy down the shoot, but rather a slight spiral around it.

A random sample of 22 shoot apices yielded the following frequencies:

Phyllotaxis	Direction	Number	Approximate %
(ca.) $3/8$ spiral.....	cathodal	7	32
(ca.) $3/8$ spiral.....	anodal	12	55
(ca.) $2/7$ spiral.....	cathodal	2	9
Bijugate (ca.) $1/5$ spiral	anodal	1	4
		22	100

Two more apices were recognized in the field as bijugate types and were studied closely because of their relative infrequency.

The $3/8$ leaf arrangement.—In the (ca.) $3/8$ spiral (fig. 1, 2a) the trace of each leaf has originated from that of the 13th older leaf below. The vascular bundles of every 13th leaf are joined with each other, as follows: 27-14-1..., 28-15-2..., etc. (fig. 1, 3a). This means that, if the leaf spiral is followed out, each orthostichy is connected by trace union with the next adjacent orthostichy. Since actually the leaf traces are not rectilinear in their upward paths but undulate slightly according to their distance from adjoining traces (fig. 4), and since the 8th and 13th leaves below a given leaf are each approximately equally spaced from the vertical plane of that leaf, it is seen that this $3/8$ phyllotaxis could as easily, or more easily, be called a $5/13$ arrangement.

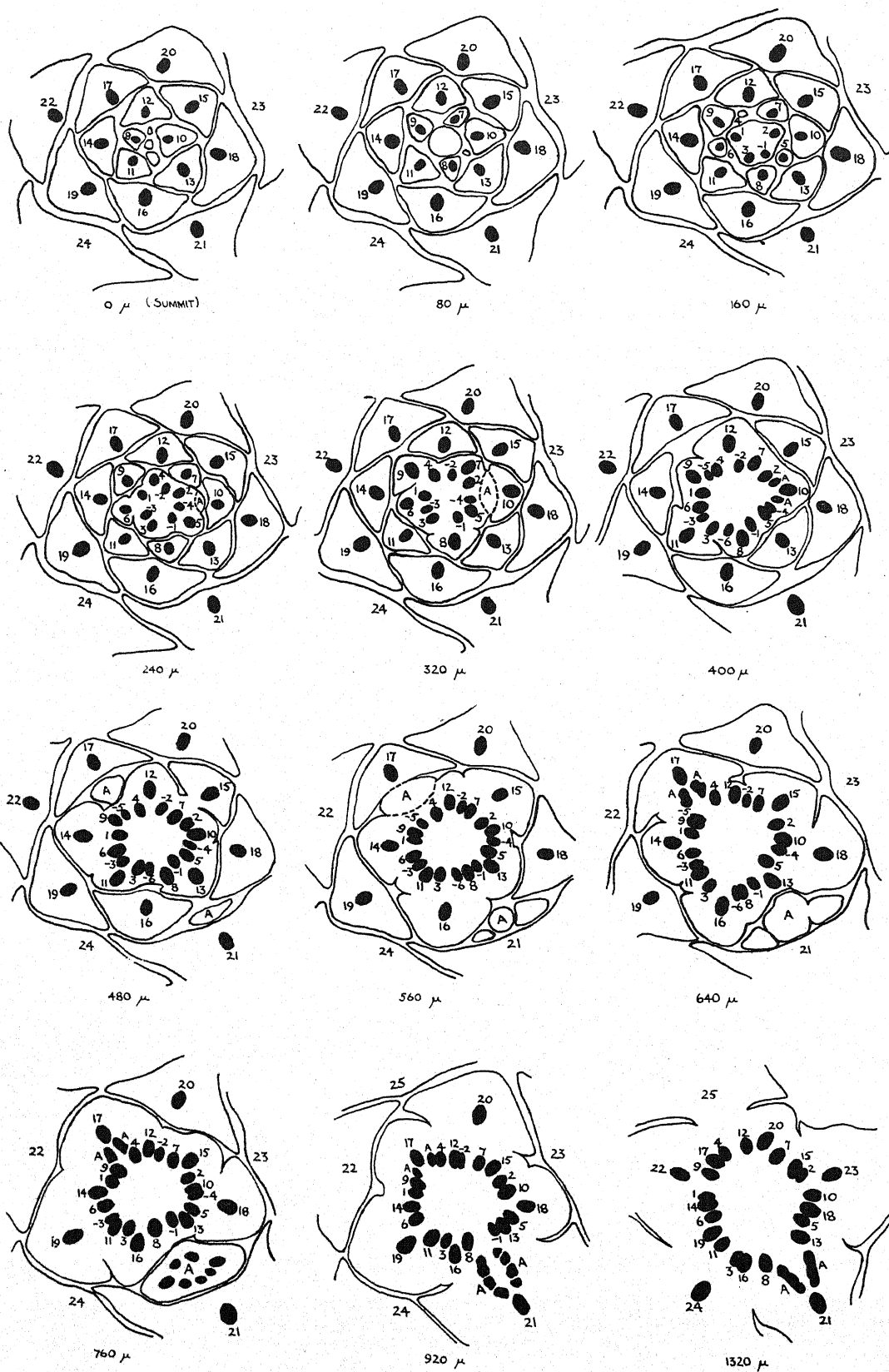
Because the geometrical symmetry of the leaves at the rosette level can be variously interpreted in terms of orthostichies and parastichies, it is the writer's opinion that the description of the various leaf and bundle arrangements in *Sequoia* can best be given by two figures:

1. The approximate divergence angle and direction between successive leaf primordia.
2. The numerical constant (sympodial number) and direction of trace linkage (table 1).

Hence the apex in figure 1 should be designated as $137\frac{1}{2}^\circ$ -anodal (in terms of its leaf arrangement); 13-cathodal (leaf trace arrangement). (Also see Esau, 1943a). The direction of trace linkage is anodal in shoots with the sinistrorse leaf spiral and cathodal in shoots with the dextrorse arrangement.

It should be noted that the angles between successive leaf primordia are approximate. There is a wide range of divergences around the theoretical angle as a mean.

The $2/7$ leaf arrangement.—In the (ca.) $2/7$ or $99\frac{1}{2}^\circ$ leaf spiral the trace of each leaf arises from



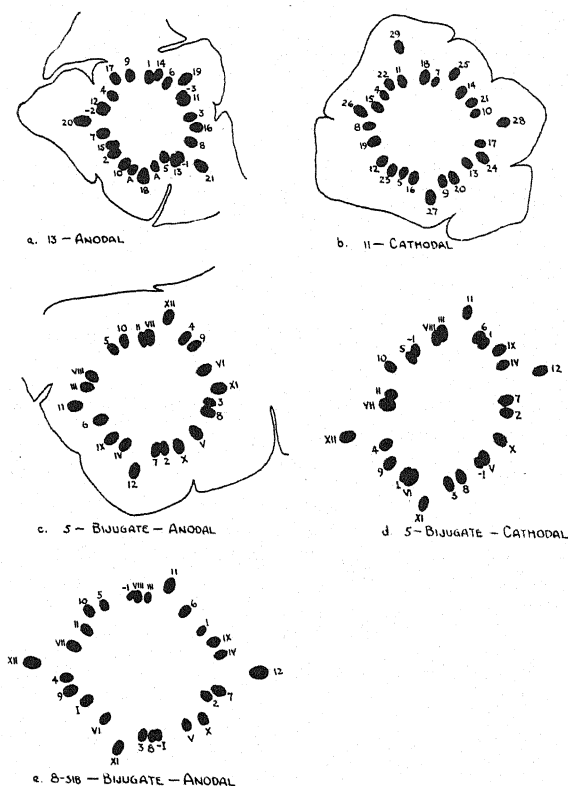


Fig. 2. Diagrammatic representation of some phyllotactic arrangements as seen in transections through various shoot apices at about 180 μ below the summit of the apical cone.

that of the 11th leaf below (fig. 2b, 3b). In the two representatives of this type of phyllotaxis, the sinistorse spiral was observed. Bundle linkage also proceeded in the cathodal direction. It is to be ex-

TABLE 1. *Types of phyllotaxis and trace union in Sequoia sempervirens.*

		Phyllotaxis		Trace union	
		Divergence	Direction	Numerical constant	Direction
Type I	a	$137\frac{1}{2}^{\circ}$	anodal	13	cathodal
	b	$137\frac{1}{2}^{\circ}$	cathodal	13	anodal
Type II	a	$99\frac{1}{2}^{\circ}$	cathodal	11	cathodal
	b	Unobserved (possible complement of IIa)			
Type III (Bijugate)	a	$68\frac{3}{4}^{\circ}$	anodal	5	cathodal
	b	$68\frac{3}{4}^{\circ}$	cathodal	5	anodal
	c	$68\frac{3}{4}^{\circ}$	anodal	8-sib	anodal
	d	Unobserved (possible complement of IIIc)			

Fig. 1. Diagrammatic series of transections through a representative shoot apex of redwood. Procambial bundles are indicated by solidly-inked oval areas. The numbers adjoining the bundles refer to the relative chronological appearance of their respective leaf primordia. Increasing values denote increasing age. Negative values refer to unformed, future leaves. The letter "A" designates axillary buds and their traces. Sections are indicated by their distances below the summit of the apical cone.

pected that the complementary type exists. See table 1.

The bijugate leaf arrangement.—The most complex, in point of bundle union, is the (ca.) $1/5$ or $68\frac{3}{4}^{\circ}$ bijugate spiral (fig. 2c, d; 3c, d, e). Incidentally, it is interesting to note that the bijugate condition for *Sequoia sempervirens* has not been reported in the literature to this writer's knowledge, nor has the $2/7$ system been mentioned in any work on this species. The bijugate type of phyllotaxis is quite similar to the decussate arrangement. It differs in that succeeding pairs of leaves are situated not at a divergence angle of 90° but at a smaller angle, so that there seems to be a $1/5$ spiral when the successive pairs are followed up the shoot.

The bijugate condition thus consists of two parallel or "sibling" primary leaf spirals. In one type, the approximate divergence angle is $68\frac{3}{4}^{\circ}$, with junction of every 5th trace in the same genetic spiral (fig. 2c, d; 3c, d). The direction of trace branching in this type is opposite to that of leaf deposition. The complements of this type were observed. The second type (with only one representative studied, fig. 3e) likewise has an approximate divergence angle of $68\frac{3}{4}^{\circ}$, but junction is with every 8th trace of the sibling spiral. Also, the direction of trace linkage in the latter is the same as that of leaf deposition (anodal here). It is

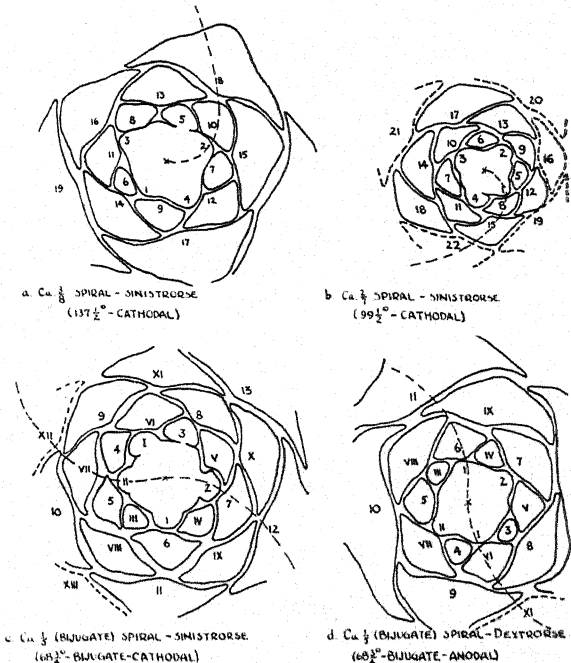


Fig. 3. Transectional diagrams complementary to those of figure 2, showing the corresponding bundle distribution in those shoots. See text.

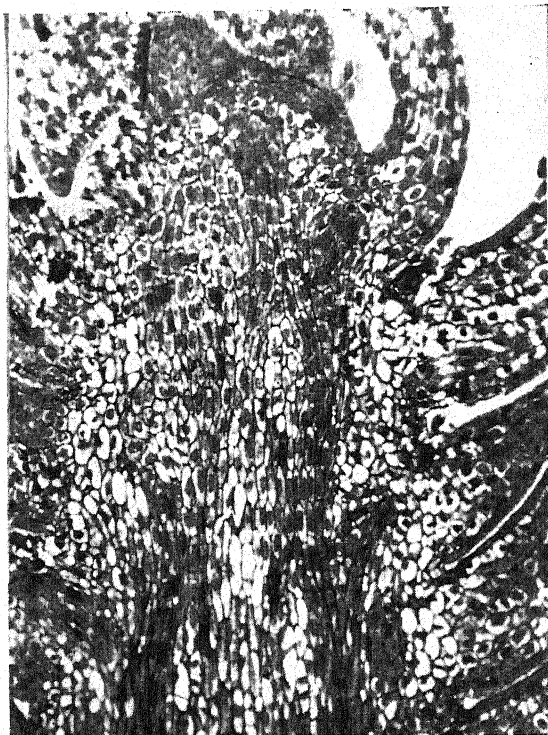


Fig. 4. Tangential longsection through an active shoot apex, showing the undulating vertical course of the vascular bundles. $\times 108$.

possible that a complementary form exists which was not discovered in this study. The type of bundle union depicted in figure 3e showed some instability, with the trace of a leaf occasionally branching from that of the fifth older leaf in the same genetic spiral.

The above descriptions are somewhat inaccurate, in that only leaf traces are considered to be present in the shoot. The arrangement of bud traces and their influence on leaf trace behavior will be considered below.

The presence of different types of bundle arrangement in *Sequoia* raises the question as to their origin. Since no transitional types have been found and sufficient data in regard to the ontogeny of the various systems are lacking, no attempt will be made to analyze the causes of this diversity. The occurrence of bijugy is interesting in connection with the dicotyledonous condition of the redwood seedling and the opposite position of the two prophylls of the bud.

BUD TRACE ARRANGEMENTS IN SEQUOIA.—The observed apices give enough stages in axillary bud formation to permit a brief review of the trace development. The bud primordium, like the leaf primordium, is already provided with procambial tissue at its earliest emergence from the flank of the apical cone (fig. 5, Sterling, 1945). This tissue consists at first of two meristematic strands arising from the traces next adjoining, situated cathodally and anodally, respectively, from the trace of the

subtending leaf in the $137\frac{1}{2}^\circ$ type (fig. 1, 3a). Meyer (1917) has stated that all axillary buds in gymnosperms are provided with two primary bundles which are linked with the anodal and cathodal traces neighboring the bundle leading to the subtending leaf. These primary bundles, at least in *Sequoia*, are the traces of the future opposite prophylls of the axillary bud. As the bud develops, the traces of other leaves of the bud develop from these primary traces at various points along their length. Reeve (1943) observed somewhat the same phenomenon in *Garrya*.

The leaf traces which branch from the prophyll traces develop at first, as seen in transection, two parallel groups of bundles, then an arc, which is finally closed to form the characteristic circle of bundles in the bud (fig. 1, at 1320μ , 920μ and 760μ).

Bud formation brings in its train some minor disturbances of the "normal" pattern of trace linkage. In shoots with the $99\frac{1}{2}^\circ$ phyllotaxis, each leaf normally derives its trace from that of the 11th leaf below. When this 11th leaf below has a bud in its axil, however, the trace of the given leaf comes from that of the 7th leaf below. Analysis of transections reveals that, in contrast to shoots with the $137\frac{1}{2}^\circ$ arrangement, the two primary bud traces are developed from the trace of the subtending leaf. There appears to be no further branching from the bundle of the gemmiferous leaf. Since the 7th and 11th leaves are fairly close to the same radial plane of this younger leaf above, it would seem that some spatial relationships of the cylinder of traces were maintained by the linkage with the 7th leaf. Crafts (1943a) also found disturbances in trace union at the level of bud emergence.

DISCUSSION.—One of the principal facts revealed by the study of vascularization is the orderliness of leaf trace branching. Even the "aberrations" introduced into this system by the presence of axillary buds seem to have an orderly sequence of trace behavior. Although the vascular arrangements may differ from shoot to shoot in a given plant, the arrangement within the individual shoot is relatively constant.

Church (1920) has suggested several inherent properties of the growing shoot that may account for this regularity of trace (and leaf) arrangement. These properties include genetically predetermined numerical factors (such as the number of trace linkage series), a factor for equal spacing of the members from each other, and a factor for physiological and anatomical localization of these members in orthogonal directions in space (localization of the traces in a cylinder through the shoot). Leaf arrangement at the shoot apex may be determined by these same factors, with the added indication that the position of leaf members in the apical rosette is partially predetermined by acropetally developing traces.

Although it might seem that leaf position could be predicted entirely on the basis of trace arrange-

ment, there are several facts making such a prediction difficult:

1. The work of Helm (1932), Snow and Snow (1931), Snow (1942), Ball (1944), and others who have performed various types of experiments on the shoot apex seems to show that phyllotaxis can be considerably varied by external factors. However, except for Helm, none of the authors has investigated in detail the vascular situation in the altered shoots. Helm's work did show the necessity of the presence of the leaf for continued differentiation in the trace of that leaf. Even here, the results of excision are questionable because of possible secondary effects of traumatic stimuli, wound chemicals, etc. (Cf. Esau's critique, 1943b, pp. 150-151.)

2. Even though it has long been recognized that opposite leaves do not arise simultaneously at the shoot apex, in one of the bijugate types investigated (fig. 2c), the disparity in chronological appearance and topographical position of the members of the youngest pairs was so great that it seemed as though a single spiral arrangement obtained at the apex. The leaf members appeared to show an independence of trace arrangement. However, too much weight cannot be placed on the behavior of a single apex.

3. In the case of shoots which have a $3/8$ leaf arrangement, but in which there are 13 sympodial bundle groups, leaf position may be conditioned by the physical relationship between the width of the leaf insertion and the circumference of the shoot. It is a well-known fact that shoots of some plants may experience a shifting between various fractions of the Fibonacci series. These shiftings may not all be accompanied by corresponding alterations in the linkage of the vascular bundles. Similarly, one of the shoots designated as "2/7" has a leaf arrangement more nearly akin to $3/11$ or $5/18$.

The theories of optimum spatial arrangement or of competing growth centers at the shoot apex (Priestley and Scott, 1933), which theories include the concept of a 120° minimum divergence angle, do not apply to two of the 22 apices selected at random. In these two, there is a divergence of approximately $99\frac{1}{2}^\circ$ (noted also by Barthelmess, 1935, in a shoot of *Cedrus Deodara*), which does not provide an equitable spatial arrangement of the leaves but which seems quite stable and well organized. It is to be stressed that the variability in phyllotaxy and trace arrangements in *Sequoia sempervirens* is not due to a shifting between the fractions of the Fibonacci series (table 1). Instead, the various systems appear to be unrelated, with little evidence for the possibility of transitions between them.

Little support can be advanced for Barthelmess' (1935) claim that the ratio of bundle width to stele diameter determines the linkage of leaf traces and their arrangement in the ring. The writer's objection is that the assumption that the leaf traces differentiate basipetally has not been borne out by

the present study. Moreover, the concept of a more or less fortuitous union of traces, implied by the theory, receives no support in the definite origin of traces from specifically situated older traces as found in *Sequoia*. However, it would seem that the formation of an axillary bud in a shoot with a $99\frac{1}{2}^\circ$ divergence could be considered as an obstructing factor causing a basipetally differentiating trace to be deflected to a different bundle from its "normal" companion, the trace of the subtending leaf.

It is the writer's opinion that, with the number of trace linkage series (sympodial constant of Barthelmess, 1935) genetically determined in the growing plant, the course of a new member of each series is fixed primarily by its spatial relation to other members of the ring. Analysis of transections appears to indicate that the new trace follows a course which would place it approximately equidistant from its two next adjacent neighbors, whatever their course, as long as they are component members of the ring. This could account for the fact that the course of an individual trace undulates in the tangential plane (fig. 4), as Geyler (1867, Plate V, fig. 1) has so well shown. (See also Crafts, 1943a.)

With regard to the phyllotactic situation in *Sequoia sempervirens*, there are two accounts in the literature: Geyler (1867), studying lateral branches, reported a phyllotaxis varying between $2/5$ and $5/13$, with emphasis on the former in his description of the bundle arrangement. Crafts (1943a), studying a single cross-sectional series of a leading shoot apex, describes a $3/8$ leaf arrangement in a cathodic spiral. He diagrams the vascular cylinder as being made up of 8 sympodial groups, with occasional "aberrant" linkages between a trace and that of the 13th leaf below. In the writer's specimens, the $3/8$ system was characterized solely by union between every 13th trace.

In view of the diversity of phyllotactic and trace linkage systems in this single species, a proper understanding of their relationship to each other and to general shoot structure could probably better be served by a study of development of plants in which the phyllotaxis varies during the course of ontogeny. Particular attention, the writer believes, should be paid to the vascular development in such a study. Investigations on experimental phyllotactic alterations should also include details of vascularization. It is hoped that the analysis of developmental differences between systems as well as of "aberrations" within a system may clarify the relationship between phyllotaxy and vascular development.

SUMMARY

The procambial strands in *Sequoia sempervirens* differentiate acropetally in the shoot apex from older strands below. The strands are always continuous, and they are present in the apex previous to the emergence of their respective leaf primordia.

The possibility is thereby indicated that the procambial strands may have a partially determining influence on leaf arrangement.

There are no cauline bundles in the redwood shoot. The bundles are common to both stem and leaf. The type of bundle linkage, and the corresponding leaf arrangement, vary in different shoots. In the same shoot, however, the manner of trace branching remains quite regular.

Various types of leaf and leaf trace arrangements have been noted, among them a $137\frac{1}{2}^\circ$ type, a $99\frac{1}{2}^\circ$ type, and bijugate forms. Complementary (i.e., anodal and cathodal) spirals are common. The description of phyllotaxy is considered best represented by the approximate divergence angle and direction of leaf formation; and leaf trace ar-

rangement by number of sympodial groups and direction of trace union.

Two procambial strands branching from leaf traces below are present at the emergence of the bud primordium. These two strands are the vascular bundles of the future opposite prophylls of the bud. The traces to the other leaves of the bud develop from the above-mentioned primary bundles.

Various theories of phyllotaxy are discussed in relation to the results of the present investigation. An attempt is made to relate leaf arrangement to vascular development in the shoot.

DEPARTMENT OF BOTANY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

- ARBER, A. 1930. Root and shoot in the angiosperms: a study of morphological categories. *New Phytol.* 29: 297-315.
- . 1941. The interpretation of leaf and root in the angiosperms. *Biol. Rev.* 16: 81-105.
- BALL, E. 1944. The effects of synthetic growth substances on the shoot apex of *Tropaeolum majus* L. *Amer. Jour. Bot.* 31: 316-327.
- BARTHELMESS, A. 1935. Über den Zusammenhang zwischen Blattstellung und Stelenbau unter besondere Berücksichtigung der Koniferen. *Bot. Arch.* 37: 207-260.
- CHURCH, A. H. 1920. On the interpretation of phenomena of phyllotaxis. Oxford Univ. Press, London.
- CRAFTS, A. S. 1943a. Vascular differentiation in the shoot apex of *Sequoia sempervirens*. *Amer. Jour. Bot.* 30: 110-121.
- . 1943b. Vascular differentiation in the shoot apices of ten coniferous species. *Amer. Jour. Bot.* 30: 382-393.
- EAMES, A. J. 1936. Morphology of vascular plants, lower groups. McGraw-Hill Book Co., Inc. New York.
- ESAU, K. 1942. Vascular differentiation in the vegetative shoot of *Linum*. I. The procambium. *Amer. Jour. Bot.* 29: 738-747.
- . 1943a. Vascular differentiation in the vegetative shoot of *Linum*. II. The first phloem and xylem. *Amer. Jour. Bot.* 30: 248-255.
- . 1943b. Origin and development of primary vascular tissues in seed plants. *Bot. Rev.* 9: 125-206.
- GEYLER, H. T. 1867. Ueber den Gefäßbündelverlauf in den Laubblatt-regionen der Coniferen. *Jahrb. f. Wiss. Bot.* 6: 55-208.
- HELM, J. 1932. Über die Beeinflussung der Sprossgewebe-Differenzierung durch Entfernen junger Blattanlagen. *Planta* 16: 607-621.
- HIRMER, M. 1922. Zur Lösung des Problems der Blattstellungen. G. Fischer, Jena.
- KAPLAN, R. 1937. Über die Bildung der Stele aus dem Urmeristem von Pteridophyten und Spermatophyten. *Planta* 27: 224-268.
- KOSTYTSHEW, S. 1922. Der Bau und das Dickenwachstum der Dikotylenstämme. *Ber. d. Deutsch. bot. Ges.* 40: 297-305.
- . 1924. Der Bau und das Dickenwachstum der Dikotylenstämme. *Beih. z. bot. Centralbl.* 40: 295-350.
- LESTIBOUDOIS, TH. 1840. Études sur l'anatomie et la physiologie des végétaux. *Ann. Sci. Nat. Bot.* II. 14: 276-314.
- MEYER, F. J. 1917. Bau und Ontogenie der Wasserleitungsbahnen und der an diese angeschlossenen Siebteile in den vegetativen Achsen der Pteridophyten, Gymnospermen und Angiospermen. *Progressus Rei Bot.* 5: 521-588.
- PRIESTLEY, J. H., AND L. I. SCOTT. 1933. Phyllotaxis in the dicotyledon from the standpoint of developmental anatomy. *Biol. Rev.* 8: 241-268.
- , ———, AND E. C. GILLETTE. 1935. The development of the shoot in *Alstroemeria* and the unit of shoot growth in monocotyledons. *Ann. Bot.* 49: 161-179.
- REEVE, R. M. 1943. Comparative ontogeny of the inflorescence and the axillary vegetative shoot in *Garrya elliptica*. *Amer. Jour. Bot.* 30: 608-619.
- SCHÜEPF, O. 1936. Untersuchungen zur Theorie der schiefen Quirle. Modelle zur Blattstellungstheorie. *Jahrb. f. Wiss. Bot.* 82: 555-580.
- SMITH, B. W. 1941. The phyllotaxis of *Costus* from the standpoint of development. *Proc. Leeds Phil. and Lit. Soc., Sci. Sect.* 4: 42-63.
- SNOW, M., AND R. SNOW. 1931. Experiments on phyllotaxis. I. The effect of isolating a primordium. *Phil. Trans. Roy. Soc. London. B* 221: 1-43.
- SNOW, R. 1942. Further experiments on whorled phyllotaxis. *New Phytol.* 41: 108-124.
- STERLING, C. 1945. Growth and vascular development in the shoot apex of *Sequoia sempervirens* (Lamb.) Endl. I. Structure and growth of the shoot apex. *Amer. Jour. Bot.* 32: 118-126.
- WEISSE, A. 1900. Sketch of the mechanical hypothesis of leaf-position. In K. Goebel's *Organography of Plants*. I. Clarendon Press, Oxford. (74-84).
- WIESNER, J. 1907. Der Lichtgenuss der Pflanzen. Wilhelm Engelmann, Leipzig.

THE MECHANISM OF COLCHICINE-INDUCED CYTOHISTOLOGICAL CHANGES IN CRANBERRY ¹

Haig Dermen ²

ATTEMPTS to induce polyploidy by means of colchicine have been markedly successful with many herbaceous plants, but have been only rarely successful with woody and semiwoody plants (Dermen and Scott, 1939; Dermen, 1941; Graner, 1941; Dermen and Bain, 1944). Furthermore, results with many herbaceous plants have indicated that all new growth following colchicine treatment may show a polyploidy condition. However, the work of Satina, Blakeslee, and Avery (1940) indicates that even in such plants not all of the tissues may become polyploidized uniformly; thus the polyploid condition in treated herbaceous plants may be confined to a sector within a particular tissue or tissues, or to one whole tissue and not to others.

Results with peach, a woody plant (Dermen, 1941), and cranberry, a semiwoody plant (Dermen and Bain, 1941, 1944), have indicated that in these plants the occurrence of the polyploid condition in all tissues resulting from colchicine treatment is probably very rare. When colchicine treatments of these plants did produce polyploidy, it was almost entirely confined to sectorial and periclinal cytochimeras from which, in the case of cranberry, various polyploid types have eventually been isolated and propagated.

It is the purpose of this discussion to consider the cytohistological basis for sectorial and periclinal cytochimeras in colchicine-treated cranberry. A clear understanding of the facts underlying these changes will undoubtedly help to explain properly some of the complexities and to overcome some difficulties encountered in attempts to induce polyploidy in plants of semiwoody and woody types. It is hoped that the discussions presented will be of particular value in working out more successful methods of identifying and isolating polyploid material from colchicine-treated plants.

The following studies are believed to have a very definite bearing on our understanding of the method by which the stem apex propagates itself, as well as of the histogenesis of primary tissues, and the ontogeny of various plant organs. It appears that induction of polyploidy by colchicine not only may result in plants of economic value, but it may also provide material for a study of many fundamental botanical problems which hitherto have been difficult of solution.

¹ Received for publication March 19, 1945.

The writer is indebted to Dr. H. A. Borthwick for many discussions with him; these had a crystallizing influence upon many aspects of the problems dealt with herein. I am also indebted to many of my colleagues for critical reading of the manuscript.

² Associate Cytologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agric. Engineering, Agric. Research Administration, U. S. Department of Agriculture.

Further studies are in progress dealing with the ontogeny of tissues in various parts of the cranberry plants. These studies are being made with different types of periclinal plants that show cytological differences either between corresponding tissues in the several plants or between different tissues in a given plant.

PRIMARY HISTOGENIC LAYERS.—Vegetative growth in a stem is primarily centered in the stem apex; more specifically, at the apical dome (fig. 1 and 2). The apical dome shown in longisectional view in figure 1 was that of an axillary bud at the beginning of its initial growth into a branch, whereas the dome shown in figure 2, also a longisectional view, was from the apex of a runner branch; both were from diploid plants. Apical domes in axillary bud and growing stem are fundamentally similar. In a mature seed the central point of growth in the epicotyl of the embryo corresponds histologically to the apical dome in the bud and stem. This seems to have been demonstrated by the cytohistological studies of *Datura* by Satina, Blakeslee, and Avery (1940).

In cranberry the cells at the curvature of the dome, especially at the highest point of its curvature, indicated by an arrow (fig. 2), are arranged in three histogenetically significant layers, designated here as *primary histogenic layers*. The number of layers at the apical dome may differ from species to species but appears to be constant and characteristic for each species. However, in some groups of plants no definite and histogenetically independent layers exist (Foster, 1939, 1941, 1943, and others).

The controlling mechanism in maintaining the constant number of histogenic layers at the apical dome lies in the mode of division of the cells of the histogenic layers. There are two principal directions of cell division: *anticlinal* and *periclinal*. By anticlinal division cells multiply and grow in a spreading manner to form a uniseriate layer, whereas by periclinal division cells are added one over another, resulting in growth in depth. In the cranberry, the cells at the very center of the first or outermost layer of the apical dome divide anticlinally; the central cells in the second layer usually divide anticlinally; and those in the third layer divide both anticlinally and periclinally. L-I, L-II, and L-III are hereafter used to designate the first or outermost, the second, and the third layers, respectively, following the usage of Satina and Blakeslee, 1941. By the anticlinal method of division in L-I and L-II a quite distinct line of demarcation appears between L-I and L-II, and between L-II and L-III at the apical dome; but because of the fact that periclinal divisions as well as anticlinal divisions occur in L-III at that region, no line of

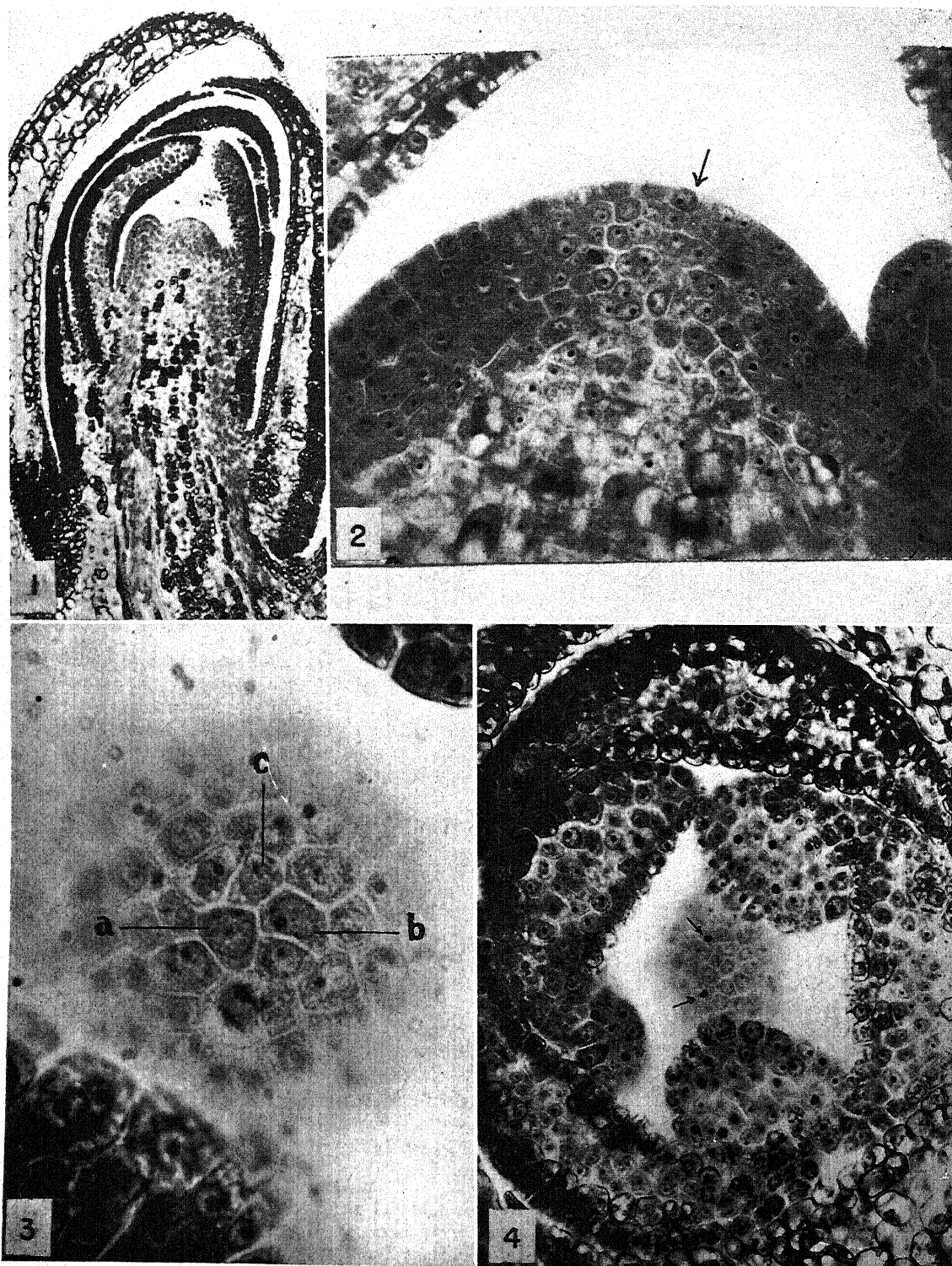


Fig. 1-4.—Fig. 1. Longisectional view of a lateral bud showing the apical dome inclosed by bud scales and young leaves. This is the stage of bud development at the time colchicine is applied in cranberry. $\times 135$.—Fig. 2. Longisectional view of the apical dome of a growing runner branch. The three uppermost layers (L-I, L-II, and L-III) in the dome represent the primary histogenic layers in cranberry. The arrow points to the central point of the dome. $\times 600$.—Fig. 3. Top or face view of the summit of the dome at the level of the outermost layer, L-I. The cells *a*, *b*, and *c* are the most central cells. The figure shows the way these cells are arranged. $\times 1200$.—Fig. 4. Two off-center cells at the dome show colchicine effect. $\times 400$.

demarcation may appear between L-III and cells to which L-III gives rise. Future studies may indicate more definitely whether the mode of cell division characteristic of each layer may ever change at the apical dome to alter the character of certain types of periclinal and histogenetic development in various parts of the plant.

CELLULAR ARRANGEMENT IN HISTOGENIC LAYERS AND THE ORIGIN OF SECTORIAL AND PERICLINAL CYTOCHIMERAS.—The cells in the primary layers at the stem apex are all meristematic and appear alike in all respects. Each cell in each layer has no doubt the same genetic potentiality, but a few cells in each layer, by their position in the curvature of the apical dome, have the function of carrying on the histogenetic continuity of each layer. These cells are those located exactly or approximately at the central point in the curvature of the apical dome. In cranberry the number of cells thus located in each layer may be one, two, or three. These cells are irregular polygons, with usually more than four sides apparent when viewed from above; and therefore, with such cellular forms, not more than three cells as a rule can be located in the very central point simultaneously and maintain a central position on the curvature of the dome as the cells divide and the stem grows forward. Therefore a tissue derived from each histogenic layer will arise from either one, two, or three cells.

As pointed out previously (Dermen and Bain, 1944), the frequent production of sectorial rather than complete tetraploidy following colchicine treatment of the apex of a growing stem or bud of cranberry indicates that not all the cells in a meristematic tissue may be affected simultaneously. In meristematic tissues generally (excluding microsporogenous tissue in the anthers at the time of meiosis), such, for instance, as those at the apical dome of the stem apex, the cells are at any one time in different stages of development. The great majority are in the nuclear phase (chromosomes still within a nuclear membrane), with only three to five per cent of cells in mitotic phases (metaphase, anaphase, and telophase) (Dermen, 1938, 1940). It is the cells at mitosis that are affected by the colchicine. Cells in nuclear phase at the time of colchicine application may become affected only if they advance to the metaphase condition while there is an effective amount of colchicine diffused into those cells.

It seems that in many herbaceous plants, where polyploidy has been total, usually all of the cells of meristematic tissues which are reached by colchicine may become affected. Presumably this is because the cells in the nuclear phase advance practically unhindered to the metaphase stage and in their turn become colchicine-affected. This may account for the preponderance of complete polyploidy in herbaceous plants following colchicine treatment.

The case with cranberry appears to be different. Preponderance of sectorial and periclinal poly-

ploidy in cranberry seems to make it evident that not all cells of the meristematic tissue in the treated area become colchicine affected. The affected cells in cranberry may include, besides those already at metaphase, those that are soon to enter the metaphase from the prophase stage. It is very likely that the cells in nuclear stages farther back than prophase are often forced to remain in a quiescent stage under what might be termed a mildly toxic effect of colchicine. This assumption is not at all improbable, considering the fact that the growth of the treated plant material is always slowed down. When colchicine has disappeared from the meristematic tissues, presumably by diffusion and dilution through the plant system, the activity of meristematic cells is revived. Consequently, at the start of new growth following treatment certain parts of the tissues will arise from the affected cells and therefore be polyploid, and other parts, arising from unaffected cells, will be cytologically unaltered.

The occurrence of polyploid sectors and the formation of periclinal polyploidy may be better understood when we take under consideration the following points: (1) the cellular arrangement in the histogenic layers, (2) the apparent histogenetic independence of the three layers, and (3) the fact of partial colchicine effect just pointed out. In figure 3 is shown the top view (face view) of the apical dome at the level of L-I. Central cells at the dome apex show up clearly, while the other cells, off-center and farther down on the slope of dome curvature, show less clearly. It appears, therefore, that the 3 marked cells, *a*, *b*, and *c* (or possibly cell *a* alone), which seem to be the most centrally located, will be the ones to perpetuate the growth of L-I during the course of growth in length of the stem. When figure 3 is examined closely, it appears that 3 cells generally touch each other at one point. It seems, therefore, that with such a cellular arrangement, usually not more than 3 cells could maintain a more or less central position at the summit of the dome. A similar situation would be expected in L-II and L-III.

Normally the character of a tissue will be the same, whether it is derived from one cell or from many cells. With polyploidy in some cells and diploidy in others, however, experimental results have shown that the two portions of the same tissue derived from cytologically different cells show certain definite changes that can be recognized, the principal recognizable changes being in size of cells and size of mature plant organs, and possibly many other features.

It appears to be purely a matter of chance whether only one cell, two cells, or three cells have a central position in each histogenic layer; consequently if one cell is most centrally located and that one happens to be polyploidized, the layer in which this cell is found and the tissue to which it will give rise will be wholly polyploid. If two cells are centrally located, and one is polyploidized, half

of the layer and half of the tissue derived from that layer will be polyploid and the other half of the layer and of the tissue will be normal. When three cells are located centrally and one or two are polyploidized, then approximately one-third or two-thirds, respectively, of the layer will be polyploid and the remaining portion will remain normal. In the same proportion, the portion of tissue derived from the layer thus cytologically altered will show polyploidy. Therefore the width of a sector of a polyploidized tissue would not be expected to be less than approximately one-third of the circumference of the cylinder or the body of a tissue. This width would be expected to be influenced by a greater or lesser rate of cell division or magnitude of cell size in the remaining portion of the tissue.

Since the histogenic layers appear to be independent of each other, and in cranberry the colchicine effect is often partial (some cells become affected and others do not), polyploidy may be induced totally or partially in one layer and not at all in the others. Whenever there is a partial effect, *sectorial polyploidy* is induced independently in each layer (a sectorial chimera occurring independently in each tissue has been named "mericlinal" by Jorgensen and Crane, 1927). When a whole layer is affected and one or two others are not, the result is a *periclinal polyploid* form, which comprises the bulk of propagated material obtained from colchicine-treated cranberry plants.

APICAL POLYPOIDY AND CONTINUITY OF POLYPOIDY.—Meristematic activity is not confined to the apical region of the stem alone, but it extends 1,000 microns or more from the apical dome back into the primordial internodal and nodal regions, and into already differentiated external and internal tissues. Consequently, it seems that when colchicine is applied to the stem apex, its polyploidizing effect may be observed in the primary histogenic layers at the apical dome and also wherever else cell division is occurring. When one, two, or all three of the central cells in the apical dome are polyploidized, the resulting polyploidy is designated as *apical polyploidy*.

Polyploidy becomes continuous or perpetual if a colchicine effect occurs in the apical central cells. Polyploidy throughout a histogenic layer indicates that the one, two, or three cells in the exact center of the apical dome have become polyploidized. In the case of sectorial polyploidy, more than one cell (either two or three) must be in the center of the apical dome in a given layer, and of these, one of the two, or one or two of the three, alone must have been polyploidized. It is, however, unlikely that the same two or three cells would perpetually remain in the exact center of the dome. Therefore, it is likely that sooner or later a little shift in position of these cells may occur, causing either a polyploidized cell or a diploid cell to lose its central position. This might transform a polyploid sector either to a completely diploid condition or to a completely polyploid condition, or change the width of the

polyploid sector from one-third to two-thirds of the total width, or the reverse. A number of such cases have actually been found to occur in cranberry. On the other hand, it is perhaps remarkable that such polyploid sectors persist as long as they do. In one case an epidermally tetraploid sector in cranberry persisted along the entire four feet of a runner.

AXIAL POLYPOIDY AND LOCALIZATION OF POLYPOIDY.—Polyploidy occurring in meristematic cells anywhere else than in the centrally located cells at the apical dome is here designated as *axial polyploidy*. Such polyploidy may be isolated or localized, except when a new apical meristem is formed in such an isolated area and develops into an axillary bud. In figure 4 two cells in the apical dome appear to have been affected by colchicine [immediate colchicine effect in meristematic cells is recognized by massed chromosomes appearing as dark dots in the affected cells under a low magnification (Dermen and Bain, 1944)]. These cells are not in the center of the dome, hence the new tissue produced by the cells that are in the center of the dome will be cytologically unaltered. The extent of polyploid tissue resulting from the affected cells would be limited and would depend upon the number of cells derived from them; and this, in turn, would be affected by the proximity of such cells to the apical center. The closer such cells are to the apical center the greater will be the number of derivatives from them and therefore the greater will be the dimensions of the resulting polyploid tissue.

CYTOLOGICAL BASIS OF CRANBERRY PHYLLOTAXY.—In cranberry, leaf primordia are initiated at points about five or six cells away from the center of the apical dome. The vertical distance between the point of origin of leaf primordia and the true apical center, as measured along the axis line, is about 15 microns. The distance along the runner axis between the two youngest leaf primordia nearest to the apical dome is about 20–30 microns. The meristematic cells in the dome region are practically isodiametric and measure about eight microns. Since the distance between the two youngest leaf primordia is about 20–30 microns, it appears that leaf primordia are initiated along the apical dome of the cranberry stem about three cells apart. The number of fully elongated cells along a mature internode in a vertical line between two adjacent leaves is about 100, and the distance between the axils of mature leaves measures, on the average, about 10 millimeters, or 10,000 microns, each cell thus averaging about 100 microns in length, an expansion of about twelve times the initial eight microns. It is indicated, therefore, that each of the three cells between the two youngest primordial leaves may divide about five times and thus give rise to 32 cells, making a total of about 96 cells, a number close to the approximate number 100, given above. Similarly, the approximate number of epidermal cells in a transection of a fully expanded internode ranges from about 200 to 350. Since the diameter of a mature stem section is about 1 to 1½

millimeters, or 1,000 to 1,500 microns, its circumference is about 3,100 to 4,700 microns; thus the average cell dimension in a cross section in a mature epidermal layer would be about 15 microns, just about twice the initial eight microns. Assuming that there is a maximum of three initial cells at the summit of the apical dome, it appears, therefore, that each of the three cells divides six or seven times to make the number of cells in a transverse section of the mature epidermis 192 or 384, approximating the 200 to 350 actually found.

Phyllotaxy of cranberry was found to be 5/13 (Bain and Dermen, 1944). If phyllotaxy in plants is of ontogenetic significance, each leaf primordium must have a morphogenetically determined locale, and the spacing between leaf primordia at the base of the apical dome must be quite precise, exclusive of any factor, artificial or natural, that would affect otherwise normal morphological development of the plant at the stem apex. In transection the number of cells in L-I at the base of the apical dome where leaf primordia originate appears to be over 20 and less than 30. On the basis of 5/13 phyllotaxy in cranberry, the number of leaves per cycle around the stem would be $13/5$ or 2.6 leaves. In two cycles there should be 5.2 leaves, in three cycles 7.8 leaves, in four cycles 10.4 leaves, and in five cycles 13 leaves. Therefore, it is probable that the number of cells at the basal periphery of the apical dome in L-I at the point of leaf initiation is 26, or approximately so, and that each leaf primordium is separated radially from the next by ten cells in the basal periphery, on the basis that there are 2.6 leaves in a 5/13 phyllotaxy. These figures concerning the number of cells and measurements of internodes and thickness of stem are variable, the growth activity at both the initiation of leaves and the expansion of the stems being influenced by environmental changes.

CYTOHISTOLOGICAL DETERMINATION OF POLYPOIDY EFFECT FOLLOWING COLCHICINE TREATMENT.—From the data presented above it becomes clear that the region of polyploidy derived from the colchicine-affected cells shown in figure 4, which are off-center, may be limited to only a short distance along a mature stem. Thus, from one cell polyploidized when off-center only one node may be found, at maturity of the stem, containing polyploid cells in one of its histogenic layers. If two such off-center, but distantly situated, cells become polyploidized and initiation of leaf primordia occurs at those points, then there will be two independent nodes showing polyploidy. If leaf primordia do not happen to originate from such cells, the polyploidy will be confined somewhere within the internodal regions. The width of polyploidized tissue resulting from each of the two cells in figure 4 will be considerably less than one-third of the stem circumference, since a width of one-third of the circumference may develop only if a colchicine-affected cell belongs to one of the three cells holding a central position simultaneously, as pointed out above.

However, the dimensions of localized sectors may at times be one-third or greater if two or more cells, which are off-center but adjacent to each other, become similarly affected.

Stem apexes of three short runners measuring 6.5, 10, and 12.5 centimeters, respectively, developing from three colchicine-treated buds, were fixed 26 days after bud treatment (Dermen and Bain, 1944) and studied cytologically. All available microsections from each runner were surveyed for metaphase face-view figures to determine directly by chromosome number, or its estimate, the extent of polyploidy throughout the apex, and thus the effectiveness of the colchicine treatment in each branch. The examination of transections extended from the apical dome back about 1,000 microns. In the cortical tissue of the 12.5-centimeter runner there were diploid, tetraploid, and octoploid cells. A little over half of the cortical tissue in a 6.5-centimeter runner was diploid; the remaining portion was mostly tetraploid, with a small diploid sector cutting the tetraploid area in two. The tissues in the 10-centimeter runner were not affected by colchicine and were entirely diploid.

In order to determine the location and extent of polyploidy in the tissues in the 12.5-centimeter runner, the position in transection and the ploidy of each cell showing a face-view metaphase, for all 87 sections cut, were recorded in the proper quadrant of the diagram presented in figure 6. Small round marks represent cells with diploid chromosome number, medium round marks cells with tetraploid number, and large round marks cells with octoploid number. The central area of the diagram represents the pith; the narrow cylinder around the pith represents the stele; the outer, wide cylinder represents the cortical tissue; and the outer circumference line represents the epidermal layer. The diagram was divided into quadrants to facilitate more or less accurate location of the diverse cytological conditions in large numbers of sections surveyed. A photomicrograph of one section, the 52nd from the apical dome, is shown in figure 5. The positions of the two figures roughly correspond with each other. Therefore, the cytological make-up indicated in the diagram is roughly similar to the true condition in the apex at the level of the section shown in the photomicrograph. The many cells at metaphase face-view showing tetraploidy were scattered throughout the serial transections of the stem, with no apparent regularity of pattern. Octoploid cells were evident at two locations, one in the cortical tissue and the other in the pith. A few diploid cells are grouped throughout the stem, making up small diploid tissue areas. This cytological situation represents true "mixoploidy," which should not be confused with sectorial and periclinal polyploidy.

The scattered distribution of the polyploid cells in the diagram in figure 6 indicates that there is not any single cytologically homogeneous sector or area occupying about one-third, one-half, or two-

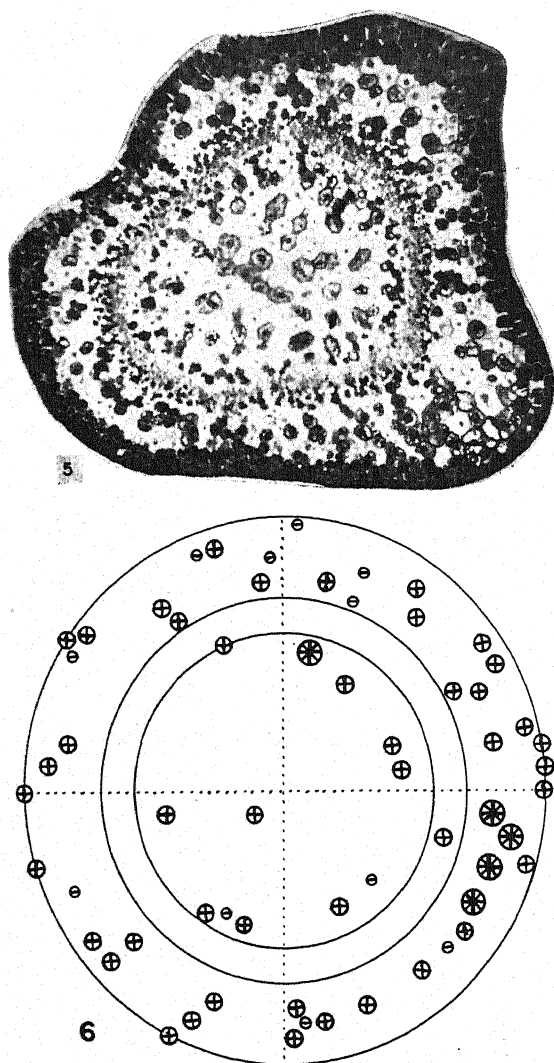


Fig. 5-6.—Fig. 5. A transection through the apex (52d section from the dome, 12μ thick) of a runner 12.5 centimeters long. It shows mixoploid condition at such a distance in the runner. $\times 90$.—Fig. 6. A diagram showing position and ploidy of cells at metaphase stage in all 87 sections cut from the apical portion of the above runner. Details in text.

thirds of any one tissue derived from any particular histogenic layer in this runner; furthermore, the number of polyploid sectors or areas is too excessive for these sectors to be of apical origin. Therefore, the polyploid sectors or isolated patches observed must have been derived from polyploidized cells located in the inner portion of the apical dome rather than at its summit. The pattern of colchicine-affected cells which could have produced such numerous and cytologically diverse sectors is illustrated in figures 7 and 8. These sections are from two buds fixed 2½ hours after colchicine treatment, one a longisectional view through the center of the bud apex and the other a transection at the level

of L-III. Under these conditions, where several adjacent meristematic cells have been affected by the colchicine, the resulting polyploid sector might be relatively wide or extend for some distance in the runner stem. In such material, prepared for cytological observation, some transections might show complete polyploidy, but its extent longitudinally in the stem would probably be very much limited, to include at most not more than one internode. Such localized polyploidy could result in apical polyploidy if a lateral bud should arise from such a polyploidized area.

METHOD OF LOCATING APICAL POLYPOIDY IN TREATED MATERIAL, AND ITS BASIS.—A consideration of the morphological changes occurring in the normal development of a lateral bud into a runner is necessary to understand the manner of determining the location of apical and axial polyploidy following colchicine treatment of the lateral bud. At the time of treatment of the lateral bud in cranberry, the length of its axis, from the apical dome to the main stem, was about 500 microns. Around the axis of such a bud, but below the dome, there were about twelve nodes. When such a bud continues its development into a runner and the bud axis has extended from 500 to 1,000 microns in length, the total number of nodes has increased from about 12 to about 16. Subsequently, when the length of the axis of the developing runner has increased to one centimeter (10,000 microns), the number of nodes will have increased to about 24; and in five centimeters to about 30 nodes. When the length of the runner axis has reached ten centimeters, only about four more nodes will have been added, making a total of about 34 nodes on such a runner. In general, as runners continue growth at a moderate rate, the length of the fully matured internode is about 1 centimeter.

In the case of the 12.5-centimeter runner previously discussed, the bud from which it developed was treated twice with one per cent colchicine solution, the second treatment two days after the first. At the time of these treatments the bud contained about twelve nodes. Mitotic activity in the bud which would be affected by the colchicine treatments would be located (1) in the one, two, or three centrally located cells of the apical dome; (2) in the off-center cells of the apical dome; and (3) in partially differentiated tissues behind the apical dome. If the centrally located cells in the apical dome of the treated bud had been polyploidized, the polyploidy in the tissues derived from the centrally located cells would have been of the apical type. If the off-center cells in the apical dome and the cells in the partially differentiated tissues had been immediately polyploidized at the time of the colchicine treatments, and the action of the colchicine had not extended beyond the time of treatment, the resulting axial polyploidy would have been confined to the first twelve nodes, or possibly to four or five nodes above the twelfth node, assuming that the additional four or five nodes were derived from off-

center cells in the dome (p. 390), where each of these cells is considered potentially equivalent to one node. Therefore the axial polyploidy would have been found through the first node at the base of the growing runner to the seventeenth node, inclusive. However, when the tip of the 12.5-centimeter runner was sectioned to a distance of 1 millimeter behind the apical dome, axial polyploidy was found to extend fully 16 nodes above the eighteenth node; whereas axial polyploidy should have extended to the eighteenth node and not beyond it. The presence of axial polyploidy in the upper region of the 12.5-centimeter runner may be explained on the supposition that the colchicine solution had penetrated into the basal portion of the treated bud, but had not reached the dome area; meanwhile, as the bud was expanding and new growth was being laid down by the apical central cells, the colchicine solution continued to penetrate the new growth.

It may be concluded that in cranberry—and, for that matter, in any other plant—polyploidy of apical origin will have to be looked for in the part of the branch directly derived from the central cells of the apical dome following colchicine treatments. In cranberry, if treatment is applied to a bud at the 12-leaf stage, apical polyploidy may be visually recognized after a branch growing from the treated bud has expanded beyond 30 or more centimeters, or when such branches have borne some 30 or more fully matured leaves. In cases where polyploidy is of sectorial nature and is derived from either the first or the second histogenic layer, its presence may be recognized by the pattern of polyploidy on or in the leaves appearing at the upper portion of the branch at certain intervals based on the 5/13 phyllotaxy in cranberry (Dermen and Bain, 1944; Bain and Dermen, 1944); hence the necessity of growing a branch of the length and with the number of leaves above indicated. The presence of sectorial polyploidy in the third layer may be more difficult to recognize. This type of chimera would affect the internal tissues of the cranberry stem alone, with which condition we have not had enough experience. When polyploidy is entire and not sectorial, its presence may be recognized in a branch that is shorter and has matured fewer leaves than above indicated.

In woody plants, when a lateral or terminal bud grows into a shoot during a growing season, this growth generally consists of an expansion of parts already present in embryonic form in a dormant bud. When colchicine treatment of a bud of such a woody plant results in polyploidy, the polyploidy in that portion of the branch derived from tissues already present in the treated bud would be of the axial type, whereas apical polyploidy would be found either in the new terminal bud or in the new lateral buds, or in both types of buds. These buds must grow into new shoots, therefore, before the presence of apical polyploidy can be detected. Unless the growth from these buds is forced in some

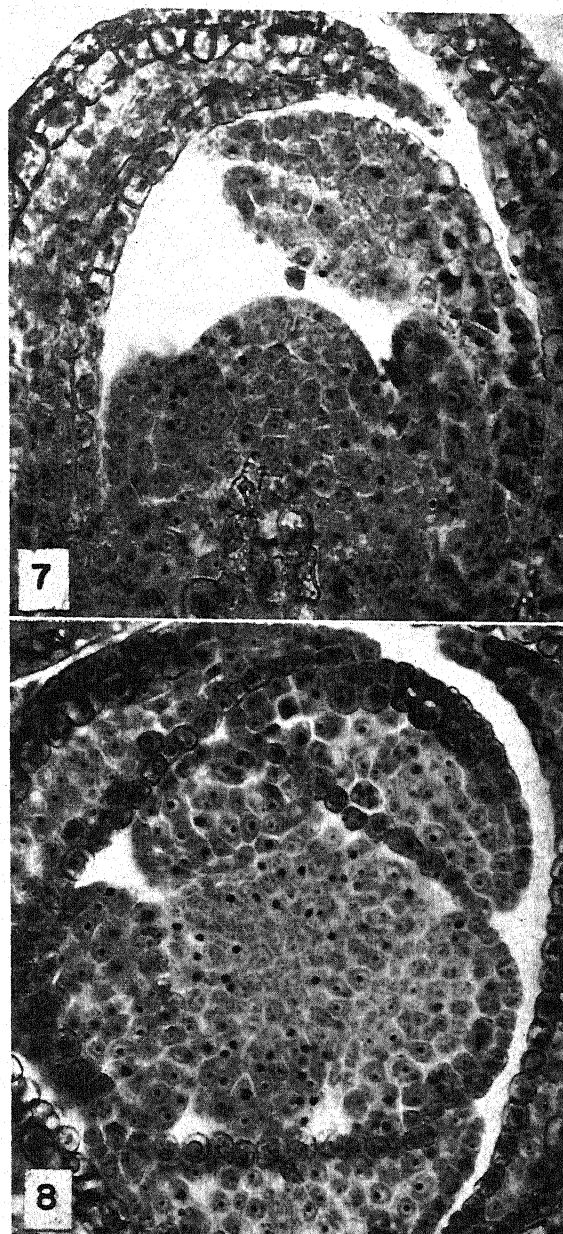


Fig. 7-8.—Fig. 7. Longisectional view of the apical dome in a bud treated with colchicine showing the pattern of colchicine-affected cells. $\times 400$.—Fig. 8. Transsectional view of the apical dome at the level of L-III shows numerous colchicine-affected cells. $\times 400$.

way, apical polyploidy may not be observed for one to three years.

SUMMARY

When cranberry plants are treated with colchicine to induce polyploidy, the results are mostly chimeric polyploids of sectorial and periclinal type. The present article reports a cytohistological study of the mechanism underlying the development of such polyploid sectorial and periclinal chimeras. In

the apical domes of runner-branch tips and of axillary buds there are three primary histogenic layers. Although the cells of these layers are alike, the layers appear to be histogenetically independent, each usually giving rise to a specific tissue or tissues. The apparent histogenetic independence of the layers seems to be determined by the direction of cell division, particularly in the cells at the summit of the apical dome. The cells of the dome in the two outermost layers, L-I and L-II, divide anticlinally, hence their uniseriate appearance, whereas those of layer III divide both anticlinally and periclinally. Thus, because of the way in which they divide, these layers seem to remain distinct and independent. Whether the anticlinal mode of division, particularly in the cells at the summit of the apical dome, sometimes changes into periclinal in either L-I or L-II is being investigated further.

One, two, or usually a maximum of three cells seem to maintain a central position in each histogenic layer at the summit of the dome, and to perform the function of carrying out histogenetic continuity of each layer. One or more of these centrally located cells in any of the three layers may become polyploidized by colchicine treatment, the other cells remaining normal. When not all of the central cells in a given layer have been polyploidized, the tissues produced from this layer show sectorial polyploidy, which may be independent in each layer. Because not more than three cells generally seem to hold a central position at the apical dome, continuous polyploid sectors might be expected to occupy either approximately one-third, one-half, or two-thirds of the circumference or mass of a stem tissue derived from a particular histogenic layer. Polyploidy may affect *in toto* one or two of the three histogenic layers, the remaining layers being normal. Such a concentric development results in periclinal polyploidy.

Polyploidy resulting from colchicine treatment

may be classified into two types: *apical* and *axial*. The former results from colchicine effect in central cells of any of the histogenic layers at the apical dome; the latter denotes polyploidy in other than central cells. Apical polyploidy results in continuous polyploidy in that portion of the growing stem resulting from those central apical cells that were polyploidized. Axial polyploidy is of localized nature and delimited in its extent. It changes to apical polyploidy only when buds arise in such localized polyploid tissue.

Some cytohistological and cytomorphological data for cranberry are presented in respect to the apical dome; the points of origin of leaf primordia; the number of leaf scales and leaf primordia in axillary buds; the number of leaf primordia, young leaves, and mature leaves at certain distances from the center of the apical dome of runner branches; and phyllotactic arrangement of leaves. These data are used to determine the location of axial and apical polyploidy in branches following bud and stem-tip treatments. In cranberry it seems that a branch arising from a treated axillary bud or stem tip should reach some 30 centimeters or more before sectorial polyploidy derived from apical polyploidy may be recognized. Complete polyploidy in one, two, or three primary histogenic layers may be recognized in shorter branches than in the case of sectorials.

It is suggested that in woody plants, if the treatment of a bud results in polyploidy, axial polyploidy will be found in the shoot of the first year's growth, whereas apical polyploidy will be found in the growth of the second or third year unless the treated bud is forced during the first year to make the equivalent of two or more years of growth.

PLANT INDUSTRY STATION,
AGRICULTURAL RESEARCH ADMINISTRATION,
U. S. DEPT. OF AGRICULTURE,
BELTSVILLE, MARYLAND

LITERATURE CITED

- BAIN, HENRY F., AND HAIG DERMEN. 1944. Sectorial polyploidy and phyllotaxy in cranberry (*Vaccinium macrocarpon* Ait.). *Amer. Jour. Bot.* 31: 581-587.
- DERMEN, HAIG. 1938. Cytological analysis of polyploidy induced by colchicine and by extremes of temperature. *Jour. Heredity* 29: 211-229.
- . 1940. Colchicine polyploidy and technique. *Bot. Rev.* 6: 599-635.
- . 1941. Simple and complex periclinal tetraploidy in peaches induced by colchicine. *Proc. Amer. Soc. Hort. Sci.* 38: 141.
- , AND HENRY F. BAIN. 1941. Periclinal and total polyploidy in cranberries induced by colchicine. *Proc. Amer. Soc. Hort. Sci.* 38: 400.
- , AND ———. 1944. A general cytohistological study of colchicine polyploidy in cranberry. *Amer. Jour. Bot.* 31: 451-463.
- , AND D. H. SCOTT. 1939. A note on natural and colchicine-induced polyploidy in peaches. *Proc. Amer. Soc. Hort. Sci.* 36: 299.
- FOSTER, A. S. 1939. Problems of structure, growth and evolution in the shoot apex of seed plants. *Bot. Rev.* 5: 454-470.
- . 1941. Comparative studies on the structure of the shoot apex in seed plants. *Bull. Torrey Bot. Club* 68: 339-350.
- . 1943. Zonal structure and growth of the shoot apex in *Microcycas calocoma* (Miq.) A.DC. *Amer. Jour. Bot.* 30: 56-73.
- GRANER, E. A. 1941. Polyploid cassava induced by colchicine treatment. *Jour. Heredity* 32: 281-288.
- JORGENSEN, C. A., AND M. B. CRANE. 1927. Formation and morphology of *Solanum* chimeras. *Jour. Genetics* 18: 247-273.
- SATINA, S., AND A. F. BLAKESLEE. Periclinal chimeras in *Datura stramonium* in relation to the development of leaf and flower. *Amer. Jour. Bot.* 28: 862-871.
- , AND AMOS G. AVERY. 1940. Demonstration of the three germ layers in the shoot apex of *Datura* by means of induced polyploidy in periclinal chimeras. *Amer. Jour. Bot.* 27: 895-905.

INTERSPECIFIC HYBRIDIZATION IN PARTHENIUM I. CROSSES BETWEEN GUAYULE (*P. ARGENTATUM*) AND MARIOLA (*P. INCANUM*)¹

Reed C. Rollins²

INTEREST IN guayule as a domestic source of natural rubber has given impetus to a wide range of investigations designed to reveal as much information concerning the species itself from as many directions of approach as possible. In looking toward the ultimate development of guayule into a crop plant of importance in the agricultural economy of North America, it seemed desirable to start early to provide the plant breeder with the widest possible assortment of genetic materials from which highly improved guayule varieties might be created. This called for the exploration of other species of *Parthenium* for desirable characteristics and the introduction of these characteristics into the genetic make-up of guayule through hybridization. Natural hybrids between guayule and mariola were observed among a large population of plants introduced from wild sources and grown in 1943 (Rollins, 1944a). The next logical step was to produce interspecific hybrids between guayule and mariola under controlled conditions. In doing this, one of the most complex situations with respect to interspecific hybridity between two species of plants known anywhere in the plant kingdom became evident. This complexity is due to several sets of uncommon conditions affecting the nature of the offspring from crosses between these two species.

In the first place, apomixis is perhaps the predominant type of reproduction, but sexuality is also common in both species. In fact a range from complete sexuality to very nearly complete apomixis has been found in each species (Powers and Rollins, 1945). Where sexuality does occur, it is not always of the usual type. Often unreduced egg cells are fertilized resulting in offspring which are triploid with respect to their immediate parents (Stebbins and Kodani, 1944). With these marked deviations from the usual type of sexual reproduction present in guayule and mariola an unusual situation involving hybridity between plant species is found. Instead of obtaining an F_1 progeny of individuals of a single general type from a given cross and its reciprocal, an appropriate cross together with its reciprocal may yield five distinct types of plants. These include: (1) F_1 maternal guayule, (2) F_1 hybrids produced from a nonreduced egg in the guayule mother plant, (3) F_1 hybrids produced after reduction in both parents, (4) F_1 hybrids produced from a nonreduced egg in the mariola mother plant, (5) F_1 maternal mariola. If we now add to the complexity of a series of interspecific progenies by including the differences due to

polyploidy in both guayule and mariola, the situation becomes highly complicated indeed (Rollins, 1944b, 1944c). Our investigations, though not complete, have progressed far enough to clarify many points heretofore obscure. One of the purposes of the present paper is to elucidate some of the facts pertinent to the picture of hybridity between guayule and mariola.

That the various effects upon the hybrids obtained through the interaction and impingement of the differences in the reproductive cycle referred to above occur in nature as well as under controlled conditions is shown by the fact that a number of wild plant types have been duplicated. Through apomixis, many first generation types, having arisen originally from crosses between guayule and mariola under natural conditions, have been preserved. Thus a direct comparison between the hybrids from wild sources and those created in the laboratory is a valid procedure.

Also, it is of high interest that comparisons may be made directly between wild material and the commercial strains which have been selected for improvement, especially in rubber content, for a number of years. None of the selected strains of guayule show marked individual morphological or growth characteristics that cannot be easily matched by material from wild sources.

MATERIALS AND METHODS.—The plants used in this study were grown from seeds obtained from both wild and domestic sources. Seeds of strains 593 and 416 were obtained from the Forest Service of the U. S. Department of Agriculture. Other plants of guayule were grown from seeds collected in Mexico by Powers or in Texas by Powers and Federer. The mariola came from seeds collected in Mexico, Texas and New Mexico.

Guayule was used as a female in eighteen attempted crosses. In twelve of these mariola was used as the pollen parent. Natural hybrids were used as the pollen parent in the other six. Twenty crosses were attempted in which mariola was used as the female parent. In every instance guayule was used as the source of pollen. Reciprocal crosses involving exactly the same plants as parents were not attempted because the small size of the plants did not provide enough flowers. However, crosses in both directions using the same collections of guayule and mariola were often made. One cross was tried in which a natural hybrid was pollinated by guayule. The number of classifiable plants and the percentages of hybrids and maternal offspring of most of these crosses have been presented in table 4 of a previous paper (Powers and Rollins, 1945).

Emasculation was accomplished in each inflorescence by removing the young disc florets well in

¹ Received for publication March 19, 1945.

² This work was completed while the author was on the staff of the Special Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

advance of anthesis. Treated inflorescences were kept isolated by covering them with small percale bags. In each instance whole flowering stalks, including a number of heads, were bagged before the ray florets were fully expanded. The effectiveness of this method of isolation has previously been shown (Powers and Rollins, 1945). Pollinations were made by inverting a dehiscing disc floret over the stigma of the chosen ray floret and bringing the anthers into contact with the stigma. Thus an ample pollen supply was assuredly placed upon the stigma of the pollinated floret.

The seeds were germinated between wet blotters in a germinator and transplanted individually into two and one-half inch pots. A mixture of pasteurized sand and soil was used as a culture medium. When the plants were of suitable size, they were transplanted to field experiments or into larger containers.

Chromosome counts were made either from acetoorcein smears of pollen mother cells or from root-tip smears. The meiotic material was fixed in a solution composed of seven parts absolute alcohol to one part glacial acetic acid. Root-tip preparations were made following the method of Dr. James R. Meyer (unpublished). Due to the difficulties frequently encountered in working with the meiotic material in some of the plants studied, particularly of the higher chromosome groups, a slight variation in chromosome number was often observed in the pollen mother cells of a given individual. Also, aneuploidy is so common in many of the higher chromosome groups, that the chromosome number of a given collection or group of hybrids varies around the expected number. Often one to several fragments or very small chromosomes have been seen. However, since the number of these fragmentary type chromosomes present was difficult to determine and their presence or absence seemed to be unrelated to the general results obtained in the crosses, they have not been included in the chromosome counts given. For these reasons the $2n$ chromosome number is often given preceded by \pm to indicate that deviations of one or two chromosomes in either direction were either observed or were expected to occur in the plants studied.

EXPERIMENTAL RESULTS.—Results from crosses in which guayule was used as the female parent fall conveniently into two categories which are correlated with the chromosome number of the female. One series of crosses gave offspring composed partly of hybrids and partly of maternal types.³ In these cases, the maternal guayule parent possessed $2n = 72-74$ chromosomes. In the other crosses on guayule, the progenies were all hybrids.

³ In general the maternal type plants were strictly like the mother plant, but there were a small number which showed that a limited amount of segregation had occurred. However, they showed no characteristics of the paternal mariola parent. It has been proposed that plants of this kind arose by pseudogamous diplospory (Powers and Rollins, 1945; Powers, 1945). Certainly they are not hybrids in the material under consideration.

The female parents in the latter crosses had $2n = +36$ chromosomes.

Guayule with 72-74 chromosomes as a female parent.—Among a total of 891 plants obtained from sixteen different crosses, 126 were found to be hybrids, the rest were maternal. As has been previously shown (Powers and Rollins, 1945) the maternal plants arose apomictically. The number of hybrids derived from given crosses ranged from 4.2 per cent up to 88.5 per cent of the total progeny. In most of the progenies, two kinds of hybrids were obtained in addition to the maternal types. One type was phenotypically intermediate between its parents, if the chromosome number of the parents was the same or nearly the same (see the third plant from the left, fig. 1); the other more nearly resembled the maternal parent and in most instances showed aberrant growth in addition (see the second plant from the left, fig. 1). The two leaves at the lower left in figure 5 are from a normal hybrid; the three leaves at the lower right of the same figure are from an aberrant hybrid of the same cross. These two hybrid types are called normal hybrids and aberrant hybrids throughout the present paper.

The terms normal and aberrant refer to the phenotypical appearance of the hybrids so classified, but the classification in reality reflects the chromosome situation in the egg cells prior to fertilization. It was found that the normal hybrids combined the reduced chromosome numbers of both parents. The aberrant hybrids in representative examples showed the full $2n$ chromosome complement of the female parent plus a reduced or 1n chromosome complement from the male parent.

When the male parent possessed an unbalanced chromosome complement such as $2n = \pm 54$ or ± 63 , there was variability in the number of chromosomes introduced into the hybrid from the pollen. This was shown in the cross ± 72 -chromosome guayule 42265-II ♀ \times 4230-IX ♂ mariola (± 54 chromosomes). Eighteen of the twenty hybrids obtained were aberrant, having arisen from the fertilization of an unreduced egg. A range of 100-104 chromosomes (± 72 from guayule and $\pm 28-32$ from mariola) was found among six plants of this group examined cytologically. This is 1-5 chromosomes higher than the expected number if only pairing and separation had occurred in the chromosomes of the male parent. The two normal hybrids possessed ± 65 and ± 66 chromosomes. Several similar crosses involving plants from different sources were less productive of hybrids. Two crosses where ± 63 -chromosome mariola plants were used as pollen parents gave hybrid progenies which also showed a variation of 2-5 chromosomes between different plants. These results are explainable on the basis of an assumption that several univalent chromosomes are present in the pollen parent and do not move in equal numbers to each pole of the spindle during meiosis. Various numbers of uni-

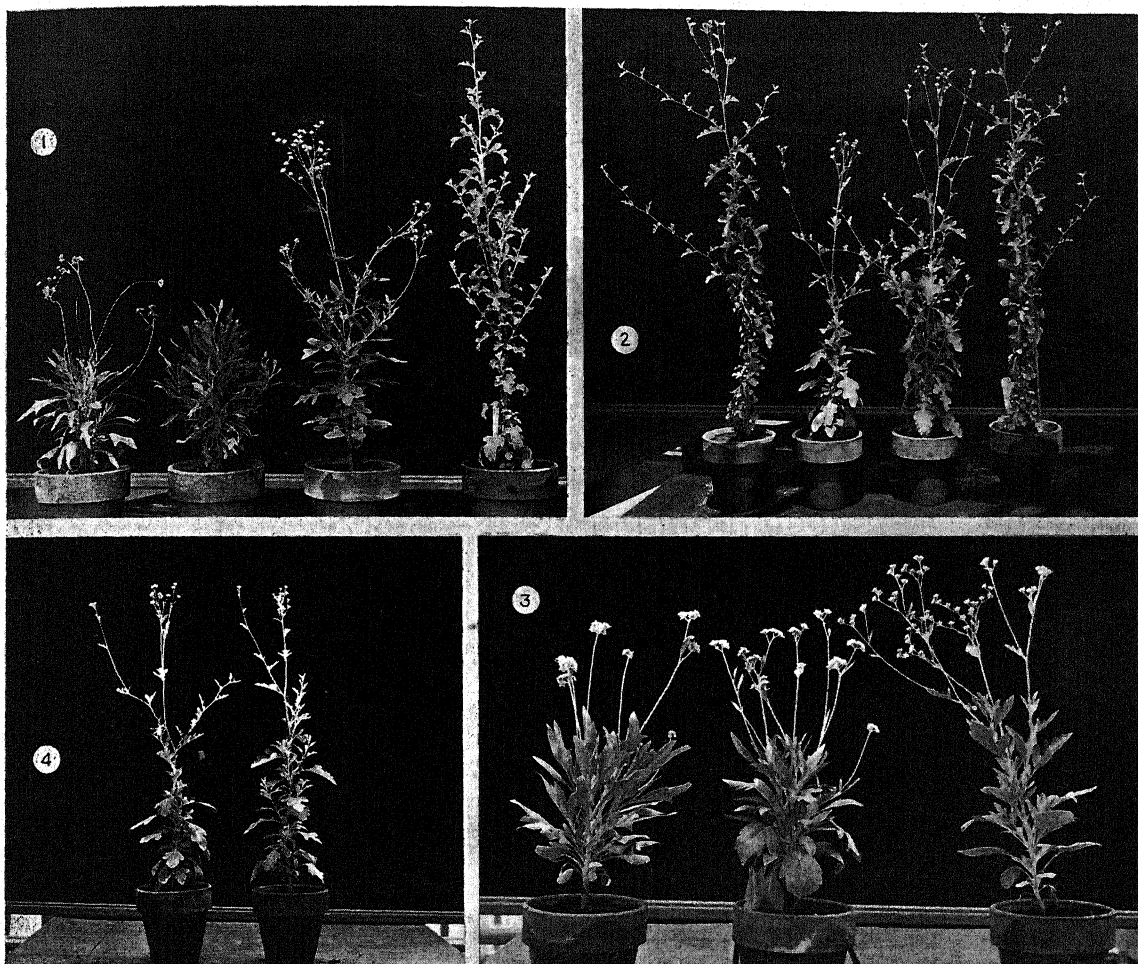


Fig. 1-4. All plants in these figures are the same age and are growing in pots which measure 4 inches in diameter. —Fig. 1. Parental types and hybrids of a cross, ± 72 -chromosome guayule \times ± 72 -chr. mariola. The plants are, left to right, (1) ± 72 -chr. maternal F_1 guayule, strain 416, (2) ± 108 -chr. aberrant F_1 hybrid, (3) ± 72 -chr. normal F_1 hybrid, (4) ± 72 -chr. apomictic F_1 from the paternal mariola parent. —Fig. 2. Maternal types and hybrids from two crosses, mariola \times guayule. The same mother plant was used in both crosses. The plants are, left to right, (1) ± 72 -chr. maternal F_1 mariola from a ± 72 -chr. mariola \times ± 72 -chr. guayule cross, (2) ± 108 -chr. F_1 hybrid from same cross as (1), (3) ± 90 -chr. F_1 hybrid from a ± 72 -chr. mariola \times $+36$ -chr. guayule, (4) maternal F_1 , same as (1). —Fig. 3. Parental types and normal hybrid of a ± 72 -chr. guayule \times ± 72 -chr. natural hybrid. The plants are, left to right, (1) ± 72 -chr. maternal F_1 guayule, strain 593, (2) ± 72 -chr. normal F_1 hybrid, (3) ± 72 -chr. apomictic F_1 from the paternal natural hybrid. —Fig. 4. The plant at the left is the same ± 108 -chr. F_1 hybrid shown second from the left in figure 2. The plant at the right is an apomictic maternal F_1 of seed collection 4260. See the text for further explanation.

valents have been frequently observed in both ± 54 - and ± 63 -chromosome plants of mariola.

Crosses involving ± 72 -chromosome guayule and ± 72 -chromosome mariola gave results comparable in many ways to those obtained when a ± 54 -chromosome mariola was employed as the pollen parent, except that the hybrids in general showed greater vigor and a lower mortality rate. As would be expected, the normal hybrids showed a greater influence from the mariola parent when the latter possessed the higher chromosome number. The aberrant hybrids from crosses of this nature gave a range of chromosome numbers of 108–112. The

normal hybrids possessed ± 72 chromosomes. In their general morphology, the aberrant hybrids are very similar to the *rough-aberrant* type plants previously described from a wild population of guayule plants (Rollins, 1944a).

Pollen of the hybrids and maternal types was examined and measured to learn something about the fertility of the hybrids and the effect of chromosome number upon pollen size. The pollen determination for each plant type was made by counting the grains which failed to stain with aniline blue occurring among a random sample of one hundred grains of each plant. In these grains the cytoplasm

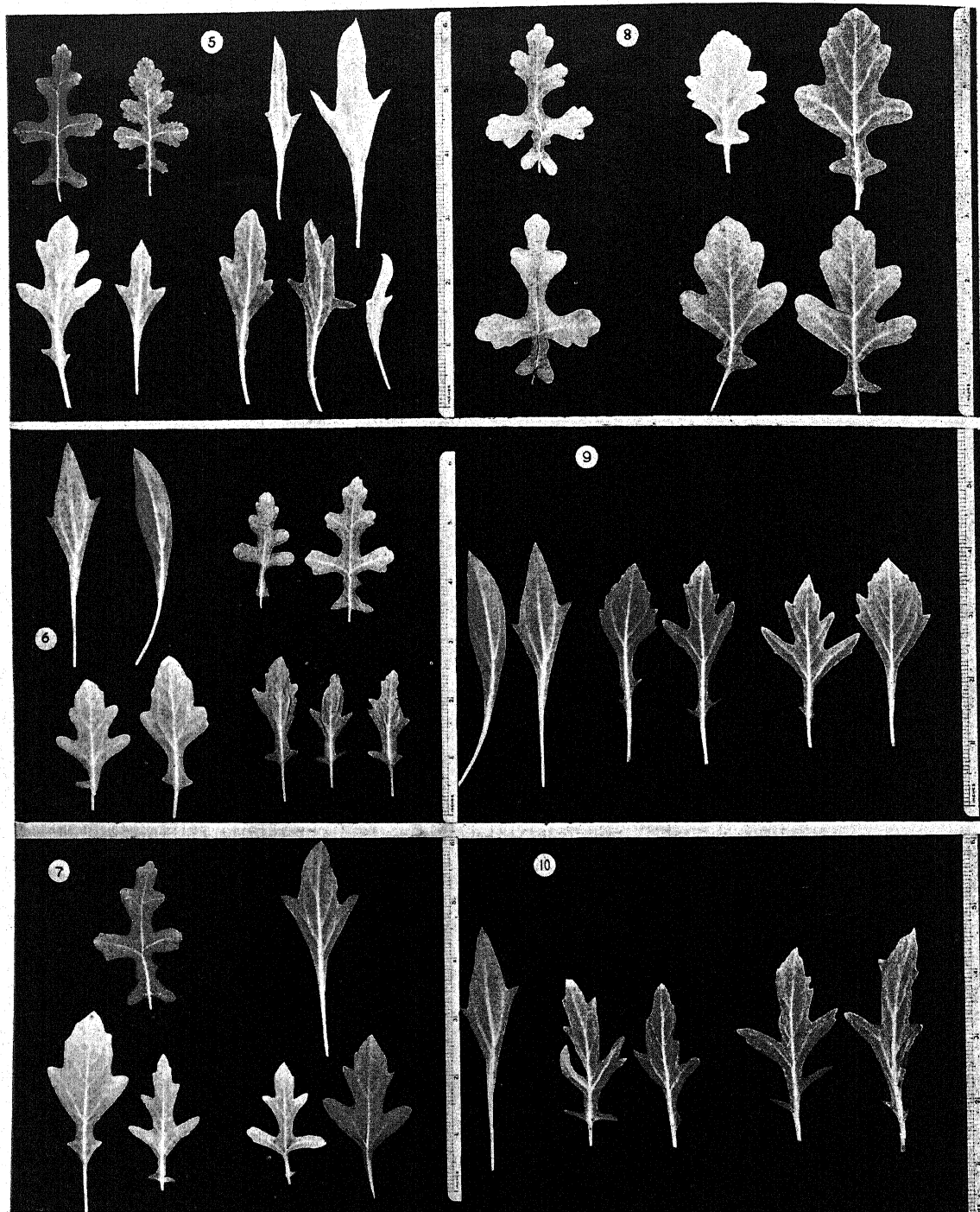


Fig. 5-10. Leaves of parental types and hybrids of a cross ± 72 -chromosome guayule ♀ \times ± 54 -chromosome mariola ♂ .—Fig. 5. Upper left, two leaves of mariola; upper right, two leaves of guayule, strain 416; lower left, two leaves of a ± 63 -chr. normal F_1 hybrid; lower right, three leaves of a ± 99 -chr. aberrant F_1 hybrid.—Fig. 6. Leaves of parental types and hybrids of a cross ± 54 -chr. mariola ♀ \times ± 72 -chr. guayule ♂ . Upper left, two leaves of guayule, strain 593; upper right, two leaves of mariola; lower left, two leaves of ± 63 -chr. normal F_1 hybrid; lower right, three leaves of a ± 90 -chr. aberrant F_1 hybrid.—Fig. 7. Comparison of leaves of a natural hybrid and a normal artificial hybrid. Upper left, leaf of mariola; upper right, leaf of guayule; lower left, two leaves of a normal F_1 hybrid; lower right, two leaves of a natural hybrid.—Fig. 8. Comparison of leaves of a wild mariola relative and an artificial hybrid. Upper and lower left, leaves of true mariola; upper right, two leaves of an F_1 hybrid in which mariola chromosomes predominate in number 2 to 1; lower right, two leaves of a wild plant from collection 4260.—

had deteriorated. The results are presented in table 1. The staining test has been used frequently to determine the amount of "good" or "bad" pollen, but it must be recognized that all of the so-called "good" pollen may not actually function as such. The data from the F_1 maternal plants given in table 1 served as a standard of comparison, since amphimixis was not involved in the production of these plants. Among the hybrids, there was considerable variation in the per cent of degenerate pollen between the different individual plants sampled both within progenies and between progenies. The average per cent given should be interpreted only as a general statement of the situation found in the groups indicated. In the normal hybrids examined, the amount of degenerate pollen is higher than in the maternal type plants. The amount of degenerate pollen is even greater in the aberrant hybrids. However, both kinds of hybrids have rela-

49.2 per cent germination obtained at the same time from two plants of guayule (strain 593). The latter percentage is high for guayule. Germinations of 40 per cent or considerably less of the seeds of field plantings of guayule is considered to be usual for the species under ordinary conditions of seed treatment (Benedict, unpublished). As might be expected, the percentage of viable seeds obtained from the hybrids is considerably lower than for guayule. However, the usefulness of the hybrids in the breeding work is not impaired on this account.

Six crosses were made in which natural interspecific hybrids were used as the pollen parent. Both aberrant and normal hybrids were produced in five of the six crosses. The three hybrid plants in a total progeny of thirty-seven plants of the sixth cross were all aberrant. In all these cases, the aberrant hybrids resembled more closely the guayule phenotype, than did aberrant hybrids from the

TABLE 1. *A comparison of pollen fertility and size in different types of plants from the progenies of interspecific crosses where 72-74 chromosome guayule plants were used as female parents.*

Type of plant	Plants sampled	Pollen grains examined and measured	Degenerate pollen grains	Size of normal pollen grains in microns				
				16.65	19.98	23.31	26.64	29.97
	Number	Number	Per cent					
F_1 maternal	38	3800	12.2	...	31.5	68.5
F_1 hybrid, normal	47	4700	19.0	4.3	78.7	17.0
F_1 hybrid, aberrant ...	52	5200	23.8	44.2	48.1	7.7

tively "good" pollen compared to interspecific hybrids in many genera where a large amount of incompatibility exists between the parent species. The decrease in size of the pollen in the normal F_1 hybrids from that shown for the F_1 maternals, reflects the smaller size of mariola pollen. The increase in size of pollen in the aberrant F_1 hybrids is correlated with the high chromosome number present in these plants. Bergner (1944), Powers (1945) and Powers and Gardner (1945) have given data on the effect of polyploidy on pollen size in guayule.

Although it was known that many of the natural hybrids produced viable seeds, it seemed desirable to run a small test upon the viability of the seeds from artificial crosses. Open-pollinated seeds of four normal hybrids and four aberrant hybrids were germinated. Several hundred seeds were used in each case and all seeds including unfilled ones were counted. The seeds from the normal hybrids gave 28.5 per cent germination while the germination obtained from the aberrant hybrids was 13.1 per cent. These percentages may be compared with

primary species crosses. The normal hybrids were intermediate between guayule and the male parent in each case and some of these were remarkably similar to the "intermediate type" which came from wild sources. This type of cross is illustrated in figure 3 which shows a ± 72 -chromosome guayule plant at the left, the daughter of a ± 72 -chromosome natural hybrid at the right and a normal F_1 hybrid with ± 72 chromosomes in the center; all plants are the same age. The guayule plant shown is a maternal F_1 . The natural hybrid is an apomict of the paternal parent used in the cross and is one of a progeny obtained by selfing.

Guayule with +36 chromosomes as a female parent.—Three crosses of this type were made and a total of one hundred and eleven plants of classifiable age were raised from them. All were hybrids. Chromosome counts of a number of hybrid plants from these crosses indicated that reduction preceded fertilization in each case. The F_1 populations showed considerable variation which appeared to be continuous between the extreme types. Regardless of the pollen parent (mariola plants with ± 54 ,

Fig. 9. Comparison of leaves of the "intermediate" wild type with those of an artificial hybrid. At the left, two leaves from a "good" guayule plant; in the middle, two leaves of a 72-chr. normal F_1 hybrid derived from a cross ± 72 -chr. guayule $\varnothing \times \pm 72$ -chr. natural hybrid σ ; at the right, two leaves of a wild "intermediate" type.—Fig. 10. Comparison of leaves of a wild rough-aberrant type with those of an artificial hybrid. At the left, a leaf of "good" guayule; in the middle, two leaves of ± 100 -chr. wild rough-aberrant plant; at the right, two leaves of a ± 101 -chr. aberrant F_1 hybrid derived from a ± 72 -chr. guayule $\varnothing \times \pm 54$ -chr. mariola σ in which nonreduction occurred preceding female gametophyte formation.

± 63 , and ± 72 chromosomes were used) the hybrids of this group of crosses consistently produced a high percentage of degenerate pollen when compared to the other hybrids. A pollen sample of one hundred grains was classified from each of ninety-two hybrid plants. Of the 9,200 pollen grains examined, 57.1 per cent were degenerate. This percentage may be compared with those given in table 1. A high percentage of "bad" pollen is known to be frequently associated with a large amount of irregularity during the meiotic process preceding pollen formation. No tabular data were taken concerning this point but the lagging of chromosomes and the failure of chromosome pairing was observed in many meiotic division figures in these particular hybrid plants.

Mariola as a female parent.—Eighteen of the twenty attempted crosses using mariola as a female parent gave a large enough progeny to be studied. Part of the detailed data giving information concerning the amount of sexuality and apomixis in the plants of mariola studied have been presented elsewhere (Powers and Rollins, 1945). Some plants were found to be completely sexual giving all hybrids when crossed with guayule. Others were completely apomictic with only maternal plants making up their immediate progenies. However, the majority of mariola plants studied reproduced largely by apomixis while giving a smaller proportion of hybrid offspring. From a total of 567 plants raised from crosses of this type, 142 were hybrids. In nearly every cross both normal hybrids and aberrant hybrids were produced. In figure 6 leaves of hybrids from a cross mariola ♀ \times guayule ♂ are shown. Two leaves of the mariola are at the upper right and two leaves of the guayule parent are at the upper left. Two leaves of the normal F_1 hybrid of this cross are shown at the lower left, while three leaves of the aberrant F_1 hybrid are at the lower right. Some normal hybrids from all of the crosses where they were obtained were examined for chromosome number. Most of them were found to have received the reduced chromosome number from both parents. Exceptions to this were found in two crosses where $+36$ -chromosome guayule plants were used as sources of pollen. Apparently the addition of $+18$ guayule chromosomes to the unreduced complement of 72 in mariola giving $2n = \pm 90$ in the offspring did not have an adverse effect. The leaves and general growth characteristics of these plants appeared to be perfectly normal. A hybrid plant of this type is shown second from the right, figure 2. However, a ± 90 -chromosome hybrid possessing ± 54 mariola chromosomes and ± 36 guayule chromosomes was invariably aberrant.

An examination of the chromosomes of a number of representative aberrant hybrids indicated that an unreduced chromosome complement had been contributed by the female parent and a reduced number by the male parent. For example,

an aberrant hybrid from a ± 72 -chromosome mariola pollinated by a ± 72 -chromosome guayule possessed ± 108 chromosomes. Figure 11 gives the situation in the crosses made. As might be expected, the aberrant hybrids were morphologically much closer to the maternal parent in each cross than were the normal hybrids from the same cross. It is interesting to compare the relatively greater effect of ± 36 guayule chromosomes in the ± 108 -chromosome hybrid (± 72 mariola plus ± 36 guayule) shown second from the left in figure 2 with the lesser effect of $+18$ guayule chromosomes in the ± 90 -chromosome hybrid (± 72 mariola plus $+18$ guayule) shown second from the right. Strictly maternal mariola plants are shown on the extreme left and right of the figure.

RECIPROCAL INTROGRESSION BETWEEN THE SPECIES.—The levels of interspecific crosses between the most representative chromosome groups of each species are summarized in figure 11. This is a simplification of the actual situation in both species and the potential crosses between them since many off chromosome numbers are known to occur. For example, one plant of mariola was found to possess ± 110 chromosomes, another over 120 and reports of ± 81 , ± 90 , 137, 142 and 144 chromosomes for guayule have been made (Powers, 1945). But so far as is now known, these chromosome numbers are not characteristic of any large natural or cultivated population and for this reason are not included in the chart.

In figure 11, the solid lines show the crosses which have been made under controlled conditions. The numbers near the center of these lines show the chromosome number group into which the hybrid offspring falls if normal meiotic reduction has occurred in both parents. These plants are normal hybrids. The numbers near the ends of these lines indicate the chromosome group of the hybrid offspring if nonreduction occurred when the parent nearest the position of the number was used as the female parent. Hybrids of this type are usually aberrant. Nonreduction in the female with subsequent fertilization is frequent above the $2n = +36$ chromosome level in both species. The numbers given are those around which the actual chromosome number varies in the interspecific hybrids with the exception of those included within parentheses. The latter are not known to occur in any plant. The broken lines in figure 11 indicate probable crosses which have occurred under natural conditions. Evidence for these has been assembled from the chromosome numbers and the morphology of natural hybrids. Part of these data have been published (Rollins, 1944a). The short broken lines radiating from the primary chromosome groups of each species indicate potential crosses which would be expected to occur under appropriate conditions. It should be remembered that in a cross where the female parent is largely apomictic, the F_1 progeny

will be largely maternal⁴ which includes, of course, the chromosome number of the mother plant.

The various combinations of chromosome numbers obtainable in crosses between these species allow for different amounts of introgression of one species into the other as a result of initial crosses between them. This is significant because results may be obtained in the F_1 generation from a single cross between guayule and mariola which would require several crosses and several generations of plants in ordinary diploid, sexual species. The situation with respect to the nature of the inheritance of apomixis in guayule and mariola has not been worked out, although Powers (1945) has postulated a sequence of steps by which apomixis may have become the predominant type of reproduction among plants of the higher chromosome groups. However, apomixis being present at the higher chromosome levels in both species allows for the perpetuation of the characteristics present in the F_1 hybrids. In all of the plants of guayule of the higher chromosome groups, none has proved to be completely apomictic. While this feature is a menace to the stability of a given strain or variety, it does allow for limited crossing and consequently the introduction of a diversity of genes. Figure 11 was designed to give some indication of the complexity of results which can be obtained by primary interspecific crosses between guayule and mariola. That back crosses of various sorts are successful has been shown. Furthermore, it is evident from a study of wild populations that plants of ultimate hybrid origin, but which are the result of a backcross or series of backcrosses, occur in nature. In fact, a series of plant types could be selected which would lead from one species by gradual steps to the other. In spite of this, guayule and mariola are good plant species by any sensible set of criteria one wishes to apply.⁵ Some of the salient points of distinction between the two species have been given previously (Rollins, 1944a). These points dealt primarily with the morphological differences. It is pertinent to point out that although the two species occur together over much of the range of guayule, mariola has a much wider natural range in both Mexico and southwestern United States. Where the latter occurs alone, at least in the places sampled, it is the pure species. Furthermore, even where guayule and mariola occur in a mixed population in many areas, the two species remain relatively pure. The main exception to this is the case of the "intermediate" type which makes

⁴ Differences between individual maternal plants of given progenies have been noted. This evidence as it bears on the nature of apomixis in guayule and mariola will be the subject of special consideration.

⁵ Interspecific crosses involving *Parthenium argentatum*, *P. incanum*, *P. stramonium*, *P. tomentosum* and *P. hysterophorus* have been made. A discussion of the interrelationships of the species of *Parthenium* and a comparison of the reproductive phenomena in this genus with similar phenomena in other genera is reserved until further data, providing a broader basis for interpretations have been assembled.

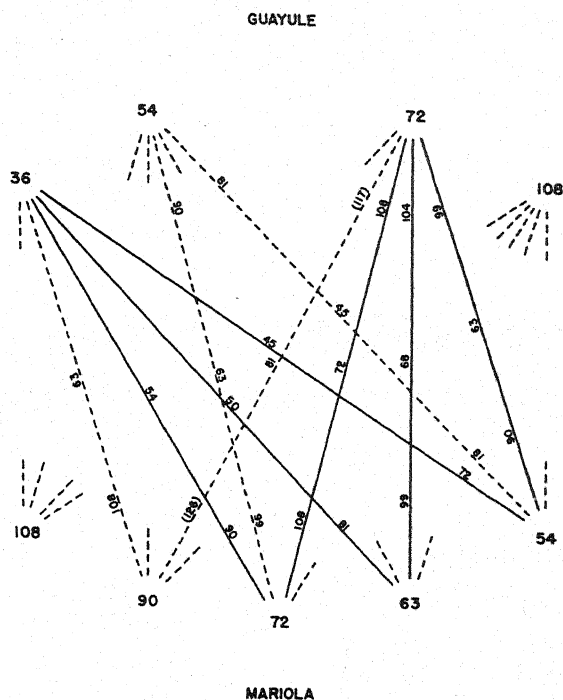


Fig. 11. Chromosome levels and extent of known crossing between guayule and mariola. The solid lines indicate crosses made in the laboratory. The broken lines indicate additional crosses for which there is evidence from wild hybrids. The bold numbers at top and bottom show the approximate chromosome number of the predominant guayule and mariola types now being grown for experimental purposes. The numbers along the lines are the approximate chromosome numbers of the hybrids from the crosses indicated. The numbers enclosed in parentheses have not actually been found. See text for further explanation.

up many large stands of shrub and obviously is the product of hybridization between the two species. However, the main point to be emphasized here is that the two species do not become swamped, losing their identity in freely interbreeding hybrid swarms. This is certainly true in the Texas guayule areas and probably holds for a large proportion of the Mexican. The natural populations of guayule have not been thoroughly studied from this point of view, particularly in Mexico. It would be surprising if areas are not present in Mexico where intercrossing occurs freely between the species. Such an area should be looked for where the sexual phases of both species come together.

ANALOGOUS PLANT TYPES FROM NATURAL POPULATIONS OF GUAYULE AND MARIOLA AND CERTAIN DERIVATIVES OF CROSSES BETWEEN THESE SPECIES.—Several plants deviating from the usual guayule type and showing some mariola-like characteristics were previously described from a wild population of guayule (Rollins, 1944a). Three of these types have been recovered from crosses between guayule and mariola. A fourth type which is largely mari-

ola, but which shows certain modifications characteristic of guayule, has also been duplicated. An explanation as to how these types arose under natural conditions can now be given.

The "intermediate" type.—The "intermediate" type which was named *Parthenium Lloydii* by Bartlett, is found extensively in Texas and Mexico and many pure stands may be seen (Powers, unpublished notes). There are moderate differences between plants of this type from the northern and southern portions of the guayule region in Texas and some of the "intermediates" from Mexico differ even more, but taken as a group they have a common stamp and seem not to differ more from each other than do plants of the usual type guayule from widely different sources. In studying the "interme-

intermediate" type previously published (table 2; Rollins, 1944a).

It is quite certain that the "intermediate" type originates by a simple backcross to guayule of normal F_1 hybrids of guayule \times mariola or the reciprocal, where complications of nonreduction, wide differences in chromosome numbers, etc., are not factors. The "intermediate" progeny thus obtained reproduces largely by apomixis and therefore does not break up by segregation as would an ordinary hybrid population. The nature of the origin of the "intermediate" type thus depicted fulfills the requirements that the process must be one which could occur fairly often at different locations and produce approximately, but not exactly, the same results each time. The differences found in the

TABLE 2. A comparison of mode of attachment and length of trichomes of different types of plants from the progenies of interspecific crosses where 72-74 chromosome guayule plants were used as female parents.

Type of plant	Plants sampled	Mode of attachment of cap cells	Trichomes measured	Mean length of cap cells
	Number		Number	Microns
F_1 maternal	25	median to slightly acentric	2500	288.11 \pm 5.07
F_1 hybrid, normal	12	near-end ^a
F_1 hybrid ^b , normal	16	markedly acentric to near-end	1690	340.85 \pm 8.89
F_1 hybrid, aberrant	22	markedly acentric to near-end	2200	396.81 \pm 7.59

^a The trichomes were too crooked to be measured accurately.

^b Representatives from three progenies in which the pollen parent was a natural interspecific hybrid are included here.

mediate" type, it appeared that this was not a single phylogenetic plant type having arisen at one place and spread widely from there. Rather, it seemed evident that the "intermediate" type had arisen independently at different times at different locations from genetically different progenitors. This conception of the nature of the "intermediate" type is supported by the evidence indicating how this type arises by hybridization between guayule and mariola. Plants resembling the "intermediate" type very closely in all morphological respects were derived from crosses of guayule \times natural interspecific hybrids. Such a cross is represented in figure 3. The similarity of this type of hybrid to the "intermediate" type is shown in figure 9. The two leaves at the right are from an "intermediate" type plant; the two leaves in the middle are from the normal F_1 hybrid of a cross, ± 72 -chromosome guayule \times ± 72 chromosome natural hybrid. Note particularly the teeth on the petiole, the leaf-shape and the lobing. For general comparative purposes, two leaves from a usual type guayule plant are shown at the left. As shown in table 2, the trichomes of sixteen hybrids from three progenies of backcrosses to guayule were studied. The average length of the trichome cap-cell of 340.85 μ compares very favorably with the average of 344.89 μ for the "in-

various populations of the "intermediate" type are due to the fact that different biotypes of guayule and mariola were involved in the original crosses.

The "intermediate" type has been relatively far more successful in nature than any other known type which traces its ultimate origin to hybridity between guayule and mariola. This must mean that whatever factors operating to the detriment of the other types in competition with the usual guayule type and other plants have been largely eliminated in the "intermediates."

The rough-aberrant type.—Plants of this type were numerous in the natural population previously studied (Rollins, 1944a). An exact count of the number present was not made. However, it is certain that they far outnumbered the plants enumerated as natural hybrids. Although the rough-aberrant type was previously thought to be of hybrid origin, the nature of the process by which it arose could scarcely have been made the subject of speculation. This was particularly true since neither apomixis nor nonreduction followed by fertilization was suspected in guayule at that time. Now, it is clear that the rough-aberrant type arises whenever an unreduced egg of a ± 72 -chromosome plant of guayule is fertilized by a mariola pollen grain. The plant second from the left in figure 1 is in reality

a rough-aberrant one although it was artificially produced. In the middle of figure 10, two leaves of a rough-aberrant plant from wild sources are shown. This plant has ± 100 chromosomes. At the right in figure 10 are two leaves of an aberrant F_1 hybrid derived from a guayule \times mariola cross. This particular F_1 has ± 101 chromosomes; ± 72 came from the mother guayule parent and ± 29 from the ± 54 -chromosome paternal mariola parent. Further evidence that the rough-aberrant type guayule is in reality an aberrant F_1 hybrid from a guayule \times mariola cross may be seen by comparing trichome lengths. In table 2 where the measurements of trichomes from twenty-two aberrant F_1 hybrids are given, an average length of 396.81μ for the cap cell is shown. This figure compares favorably with the average of 412.96μ previously published (table 2; Rollins, 1944a) for a group of twenty plants of the rough-aberrant (rough) type.

It would be a mistake to assert that the rough-aberrant type represented a homogeneous group of plants. There are marked differences between plants of this type and the distinctive features of each are due to the differences in the hereditary nature of the parents involved. However, as in the "intermediate" type plants, the rough-aberrant type shows an array of characters which are common to all.

No native stands of the rough-aberrant type are known. Nevertheless, tests on several rough-aberrant plants have shown that these plants set viable seed and reproduce largely by apomixis. A small progeny from each of five different aberrant F_1 hybrids has been grown to classifiable age. In all cases the progenies were largely maternal, showing that these plants also reproduced mostly by apomixis. These tests would seem to indicate that rough-aberrant plants ought to be found in the wild at least occasionally, although it is doubtful whether this type of plant could compete successfully on a large scale. Certainly the vigor of these plants is greatly impaired by the effects of the high number of chromosomes present in them (see Stebbins and Kodani, 1944; Powers, 1945).

The normal hybrid.—The normal hybrid made up less than one-tenth of one per cent of a 205,000-plant population of guayule whose seeds came from wild sources. Crosses between guayule and mariola have shown that this type of plant arises when normal reduction and fertilization take place. A comparison may be made between two leaves of a wild normal hybrid at the lower right, figure 7, and an artificial normal hybrid at the lower left. A leaf of mariola (upper left) and guayule (upper right) are shown for general comparative purposes. A study of many plants from these separate sources shows that their general morphology is similar throughout. Of course, many differences of a minor nature may be seen between different plants. Normal hybrids occur in nature, but no large stands have been found. Whether the normal hybrid reproduces largely by apomixis or by sexual means depends upon the parentage. Controlled crosses

have shown that several wild hybrids which came from Texas where no highly sexual forms of either guayule or mariola have been found, reproduced largely by apomixis. On the other hand, of two wild hybrids from the state of Durango, Mexico, one gave what appeared to be a wholly apomictic progeny, while the other gave a progeny composed almost completely of hybrids. Progenies of three normal F_1 hybrids have been grown. The results were not uniform. One plant whose parents were both known to be sexual gave a highly segregating population, all of the individual plants being hybrids. Two plants where one parent or both were known to be highly apomictic gave progenies which were almost wholly maternal, hence they were apomicts. The implication from these results is that whether a normal hybrid reproduces apomictically or sexually depends upon the genes affecting this phenomenon received from its parents. As Powers (1945) has shown, several independent steps are apparently involved in the conversion of a sexually reproducing plant type to one which reproduces apomictically.

Collection 4260.—Seed collection 4260 is of interest because the plants raised from it are not true mariola and because the general type of plant represented by this collection was recovered from mariola \times guayule crosses. In a notation made at the time collection 4260 was made, Powers (unpublished notes) says, "Dr. (W. B.) McCallum states that these plants are not *Parthenium incanum* (mariola) but are from a very closely related species." *P. Lozanianum* Bartlett is reputed to be closely related to mariola, but the validity of this species has not been verified, nor have our plants been compared with representative material of this species. The plant at the right in figure 4 is an apomictic F_1 of a plant from collection 4260 and is to be compared with the plant at the left which is an F_1 hybrid from an interspecific cross in which a ± 72 -chromosome mariola was used as the female parent. The plant at the left has ± 108 -chromosomes, indicating that the egg of the mariola parent did not possess the reduced number of chromosomes. Hence this hybrid has a predominance of mariola chromosomes. This accounts for its close affinity to mariola. Two leaves each of the two plants shown in figure 4 are shown in figure 8. The two at the upper right are from the F_1 hybrid; those at the lower right are from the maternal daughter of a wild plant of 4260. Two leaves of mariola are at the left in figure 8 for comparative purposes. It is not contended that the F_1 hybrid having a 2 to 1 ratio of mariola to guayule chromosomes is an exact duplication of the type of plant in the wild of which 4260 is representative. Rather this shows the way in which plants of this sort probably arose through hybridization.

POSSIBLE UTILIZATION OF HYBRIDS BETWEEN GUAYULE AND MARIOLA.—Certain factors influencing the rubber-yielding capacity and adaptability of guayule can certainly be radically altered by the intro-

duction of genes from mariola which affect its general physiology and growth characteristics. Whether these alterations are favorable or unfavorable to the accomplishment of the desired result can only be determined by experimentation. Mariola as a species has certain characteristics which appear to be desirable from the point of view of the ultimate improvement of guayule as a domestic rubber-producing plant. Some of these are: more winter hardiness than guayule, faster growth during the early years of growth thus obtaining greater size in a shorter time, greater resistance to certain diseases, and the production of several to many canes from the crown instead of a short single trunk as in guayule. The latter characteristic may be important if pollarding becomes a method of harvest for the shrub. That any one or all of these characteristics may be wholly or partially introduced into guayule by introgressive hybridization seems highly probable. There is no question but that hybrids of many sorts can be obtained at will. These are both vigorous and fertile. The success of the "intermediate" type in nature and the greater vigor and size of this type plant when compared to the usual guayule type under cultivation clearly shows that interspecific hybridization can and should play an important role in the development of guayule as a crop plant.

SUMMARY

The progenies of thirty-six crosses between guayule and mariola have been studied. Each species was used as the female parent in an equal number of successful crosses. Guayule plants with $2n = +36$ chromosomes were completely sexual and when used as the female parent their progenies consisted wholly of hybrids. The ± 72 -chromosome guayule plants used reproduced largely by apomixis, but one plant gave 38.5 per cent hybrids. All the crosses gave at least a few hybrids. Single F_1 progenies of many crosses gave three types of plants, namely, maternals, normal hybrids and aberrant hybrids. The maternals arose by apomixis (non-reduced pseudogamy and possibly pseudogamous diplospory)⁶; the normal hybrids were the result of normal reduction in both parents with

⁶ See footnote 3.

subsequent fertilization (normal sexuality); and the aberrant hybrids arose when nonreduction in the female was followed by fertilization (abnormal sexuality).

In addition to being affected by the method of reproduction operative, the nature of the hybrids between guayule and mariola is affected by the balance of the chromosomes of one species to the other. The fact that a long polyploid series is present in both species allows for a wide interplay in ratio of guayule to mariola chromosomes. Furthermore, the number of combinations obtainable in the F_1 offspring is greatly enhanced by the fact that both reduction and nonreduction occur at each chromosome level except in the purely sexual forms. So far as the investigations have gone, crosses are compatible in any and all of these situations. The complexity thus obtained in the picture of hybridity resulting from primary crosses between guayule and mariola is unusual in the plant kingdom.

Three plant types previously described from a large population of guayule obtained from wild sources and determined as having certain mariola-like characteristics have been approximately duplicated by artificially produced hybrids. A fourth wild type which is nearly related to mariola has been matched by analogous F_1 hybrids from controlled crosses. Thus an explanation of the origination of these distinguishable wild types has been worked out. That a good deal of crossing between guayule and mariola occurs in nature must go unquestioned. In spite of this, guayule and mariola remain as good plant species and may be found predominantly in the pure form even where they intermingle freely.

Considering the ease with which hybrids may be obtained between guayule and mariola and the fact that mariola possesses characteristics which will probably prove to be of value in improving guayule as a rubber producing crop-plant, there appears to be a definite place for interspecific hybridization involving these two species in the breeding program concerned with guayule improvement.

SCHOOL OF BIOLOGICAL SCIENCES,
NATURAL HISTORY MUSEUM,
STANFORD UNIVERSITY, CALIFORNIA

LITERATURE CITED

- BERGNER, A. D. 1944. Guayule plants with low chromosome numbers. *Science* 99: 224-225.
- POWERS, LE ROY. 1945. Fertilization without reduction in guayule (*Parthenium argentatum* Gray) and a hypothesis as to the evolution of apomixis and polyploidy. In press.
- , AND ELDON J. GARDNER. 1945. Frequency of aborted pollen grains and microcytes in guayule, *Parthenium argentatum* Gray. *Jour. Amer. Soc. Agron.* 37: 184-193.
- , AND REED C. ROLLINS. 1945. Reproduction and pollination studies in *Parthenium argentatum* Gray and *P. incanum* H. B. K. *Jour. Amer. Soc. Agron.* 37: 96-112.
- ROLLINS, REED C. 1944a. Evidence for natural hybridity between guayule (*Parthenium argentatum*) and mariola (*Parthenium incanum*). *Amer. Jour. Bot.* 31: 93-99.
- . 1944b. Some known and probable levels of reciprocal introgression between guayule (*Parthenium argentatum*) and mariola (*P. incanum*). *Records Genetics Soc. Amer. No. 13*: 31-32. Abstract.
- . 1944c. Analogous plant types from natural populations of guayule (*Parthenium argentatum*) and mariola (*P. incanum*), and certain derivatives of crosses between these species. *Amer. Jour. Bot.* 31: 138-148. Abstract.
- STEBBINS, G. LEDYARD, JR., AND MASUO KODANI. 1944. Chromosomal variation in Guayule and Mariola. *Jour. Heredity* 35: 161-172.

STUDIES ON CHLORELLA VULGARIS. X. INFLUENCE OF THE AGE OF THE CULTURE ON THE ACCUMULATION OF CHLORELLIN¹

Robertson Pratt, John F. Oneto, and Jane Pratt

CHLORELLIN, AN antibiotic substance or complex of substances active against a number of Gram-positive and Gram-negative bacteria has been extracted from cultures of *Chlorella* in inorganic nutrient solutions (Pratt *et al.*, 1944) and some of its biological properties have been described (Pratt, 1942, 1943).

To date, however, the yields of chlorellin have been relatively small. The present study was undertaken, therefore, to ascertain the course of accumulation of this antibiotic agent in growing cultures of *Chlorella vulgaris* with a view to determining the age at which the maximum quantity of the active compound may be obtained from the external solution.

MATERIALS AND METHODS.—The organism used in these studies was *Chlorella vulgaris*. The cells were cultured as described previously (Pratt, 1943) in 500 ml. Florence flasks, each of which contained 300 ml. of nutrient solution. The initial density of population in each culture was 100 cells/cu.mm.

At suitable intervals during a period of nearly two months, duplicate cultures were prepared for testing as follows:

1. Cells removed by filtration of culture through No. 5 Whatman paper.
2. Volume of clear filtrate reduced to about one-tenth by distillation under reduced pressure (temperature did not exceed 35°C.).
3. Residue from No. 2 evaporated to dryness over CaCl_2 in evacuated desiccator.
4. Residual salt cake extracted with 10 ml. CHCl_3 which had been redistilled recently from K_2CO_3 .
5. Suspension from No. 4 filtered through No. 5 Whatman paper.
6. Filtrate from No. 5 evaporated to dryness *in vacuo* without heating.
7. Ten ml. one per cent potassium phosphate buffer (pH 7) added to residue.

The solutions that were obtained were tested for antibacterial activity by the cylinder plate method (Abraham *et al.*, 1941) using *Staphylococcus aureus* NRRL strain No. 313 (same as F.D.A. strain No. 209) as the test organism and an incubation period of 18 hours at 37°C. Extracts prepared from blank cultures which were not inoculated showed no antibiotic activity. The diameter of the cylinders employed in these tests was 8 mm. Therefore, a preparation lacking antibiotic activity (as measured by this test) is indicated by a value of 8 mm.

RESULTS.—The principal results from the first group of experiments are shown in figure 1. The solid curve in the lower portion of the figure shows that the increase in cell number followed the normal course that has been described previously. The

dotted sigmoid curve, representing the idealized growth curve for *Chlorella* (Pratt, 1940), is drawn according to the equation

$$\log \frac{X}{A-X} = K(t-t_1) \quad (1)$$

where X represents the density of population at time, t ; A represents the maximum density of population that is attained; t_1 represents the time when $X = A/2$; and K is a constant. Numerical values for the terms in the equation are from earlier work (loc. cit.). The solid curve in the upper portion of figure 1 represents the diameters of the zones of inhibition that extracts from the cultures of different ages caused on plates of *Staph. aureus*.

As was anticipated from earlier work, it was found that solutions from cultures that have attained their full growth—i.e., two weeks of age or older under the conditions of these experiments—are relatively rich in chlorellin. The entirely unexpected result was the discovery that almost as much chlorellin can be extracted from very young cultures—i.e., cultures about 2 days old. Between the second and sixth days the chlorellin content of the cultures decreased rapidly and then increased abruptly from the sixth day until about the twelfth or fourteenth day after which time it remained at approximately the same relatively high level. It is noteworthy that the period of rapid decrease in chlorellin concentration coincided with the phase of growth during which the rate of growth was continuously increasing and that the lowest concentration coincided approximately with the time of most rapid increase in density of population. This relation is emphasized by comparison of the curves for growth and for diameter of zones of inhibition with the dotted curve in the center section of figure 1. The time axis is the same for all curves. The dotted curve in the center section is drawn according to the equation

$$\frac{dx}{dt} = kx(A-x) \quad (2)$$

and represents the differential form of equation (1).

To verify the observation that the concentration of chlorellin in the external solution decreases during periods of rapid increase in population, the following experiment was performed. Cells were removed by centrifugation from several thirty-one-day old cultures of the same series, and the cell-free solutions were pooled.² To this 1500 ml. pool of pale yellow solution 20 Gm. Norite "A" were added and the mixture was stirred for 20 minutes. Then the carbon was removed by filtration. A small portion of the

² After the normal maximum density of population has been attained (ca. 95,000–100,000 cells/cu. mm. under the conditions of these experiments) the population remains relatively static for several weeks.

¹ Received for publication May 10, 1945.

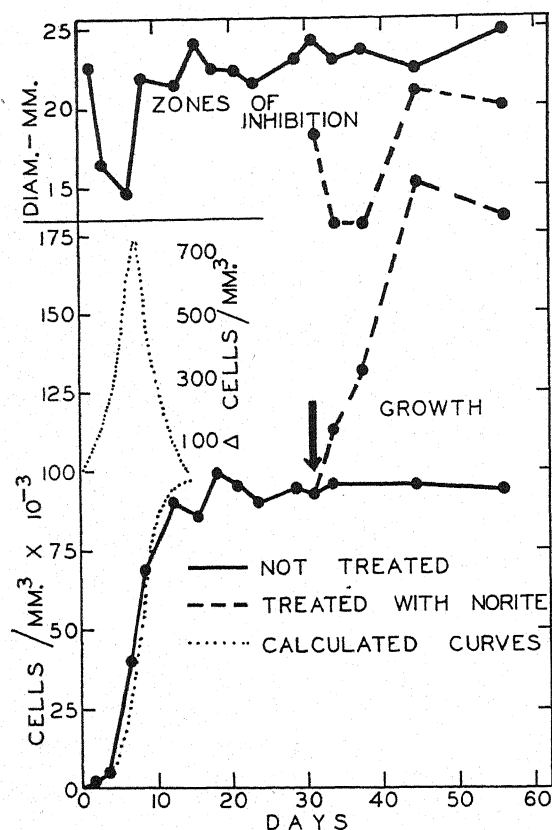


Fig. 1. (Upper portion.) Diameter of zone of inhibition caused by extracts from cultures of different ages on plates of *Staph. aureus*. A value of 8 mm. indicates no zone of inhibition, since that is the diameter of the standard cylinder. (Center portion.) Increase in population of *Chlorella vulgaris* cultures as a function of time—data from Pratt (1940). (Lower portion.) Population in *Chlorella vulgaris* cultures of different ages. Dotted curve represents theoretical normal growth curve—data from Pratt (1940). The arrow represents time at which Norite treatment was used. See text for explanation.

clear, colorless filtrate that was obtained was used to prepare a suspension of the cells that had been removed from the original cultures, another portion was set aside for extraction and the remainder was dispensed in the culture vessels (300 ml./flask). A sufficient volume of the cell suspension was added to each flask to give a population of 2.79×10^{10} cells per flask which was equivalent to the mean population in the cultures at the time of the treatment. The time at which cultures were subjected to this treatment is indicated by an arrow (fig. 1).

The effect of this procedure on subsequent density of population in the cultures and on the antibiotic activity of extracts prepared from them is shown by the dashed curves. First, as has been described previously for cultures treated in this manner (Pratt, 1944) there was a renewed activity of growth, the course of which tended to duplicate that of the first growth period. Concomitant with this

there was a slight rise in the pH value of the solutions. The curves in the upper portion of figure 1 show that the Norite treatment removed an appreciable portion, although not all, of the antibiotic material from the solutions. The significant observation is the fact that again a decrease in antibiotic activity accompanied the first part of the growth cycle and that as the rate of increase in population declined there was an increase in the antibiotic activity of the extracts prepared from the different solutions.

In another set of experiments two series of cultures were inoculated. Series A was inoculated in the regular way and served as a control. Series B was inoculated with cells which had been soaked for one hour in two changes of distilled water immediately prior to their inoculation into the culture. Thus, cells of this series should have been virtually devoid of chlorellin. The results of the experiments are shown in figure 2. It is noteworthy that in the cultures of series B the initial rise in chlorellin concentration of the external solution during the first 36 hours was lacking and that the curve representing antibiotic activity of the extracts from the solutions of this series remained at a relatively low level throughout the first half of the growth period, the time during which the rate of increase in density of population was continuously accelerating. During the latter half of the growth cycle when the rate of increase in density of population was declining, the concentration of chlorellin in the external solution increased.

DISCUSSION.—The facts observed in these experiments are that when cells from a four-day old culture of *Chlorella vulgaris* are placed directly in fresh nutrient solutions there is a rapid increase in the chlorellin content of the external solution during the first thirty-six to forty-eight hours followed by a sharp decline during the next two to four days. The period of this decline is concomitant with the period during which the rate of increase in density of population is continuously accelerating and the time at which the lowest concentration of chlorellin is present in the external solution seems to coincide approximately with the peak of the differential growth curve. Following this stage, and coincident with the period of continuously diminishing rates of increase in density of population is a period during which the chlorellin concentration in the external solution increases. When, however, the inoculum consists of cells that have been soaked in distilled water to remove the chlorellin, the initial increase and subsequent diminution in chlorellin content of the external solution are eliminated. Only the final increase in concentration that accompanies the declining rate of growth in the later stages occurs.

It is possible to make several interpretations of these facts that are consistent with the present data and with previously published reports. It has been suggested in previous work that chlorellin is toxic for cells of *Chlorella* as well as for certain other

organisms.³ Therefore, it may be hypothesized from the present experiments that if this is true, actively growing and dividing cells are capable of destroying, inactivating, or detoxifying chlorellin, but that static (non-dividing) cells possess this ability only slightly, if at all. Therefore, when only a small fraction of the cells in a culture is dividing, production of chlorellin exceeds the detoxifying or inactivating capacity of the cells and a surplus is available to diffuse from the cells and to accumulate in the external solution. This condition prevails in the cultures during the first two days following inoculation. Evidence already in the literature shows that chlorellin diffuses readily through cell membranes and is probably distributed between the cells and external solution according to the concentration gradient (Pratt, 1942, 1944).

During the next phase of development when a very large fraction of the cells is dividing actively and the actual numbers of cells in the colony is increasing very rapidly, the detoxifying or inactivating capacity of the culture is large and chlorellin disappears from the external medium. Later, when the rate of increase in density of population is declining, an ever increasing proportion of the cells becomes static and divides seldom. At this time chlorellin is again produced in excess and so accumulates once more in the cells and then diffuses into the external solution.

If, on the other hand, later studies should show that chlorellin is an essential metabolite for cells of *Chlorella vulgaris*, the observations recorded in the present work could be accounted for with equal facility. The data in that case could be taken to indicate that only static or non-dividing cells produce chlorellin in excess of their physiologic needs. Therefore, it would be only when cells were dividing slowly or when a relatively small proportion of the cells in the culture was dividing that production of chlorellin would exceed current demands and that a surplus would be available to diffuse from the cells and to accumulate in the external solution. This would apply to the two days immediately following inoculation. During the second period of development the production of chlorellin might well fall short of current requirements and all or some of the previously accumulated chlorellin might be metabolized by the very rapidly increasing number of new cells, thus causing its disappearance from the culture solution. During the final stages of the growth curve, chlorellin would accumulate again because of the increasing proportion of static, relatively inactive cells in the culture.

Another factor that should not be neglected is the possibility that changes in the permeability of the cells occur with increasing age of the cultures. Maximov and Mozhaeva (1944) reported that the aging process in cells of oats and broadbeans is characterized first by reduction and later by an increase in permeability of the protoplasm. In colonies of *Chlorella vulgaris*, more than three days old, the number

³ Exceedingly low concentrations may be stimulating for *Chlorella*. (See Pratt, 1942.)

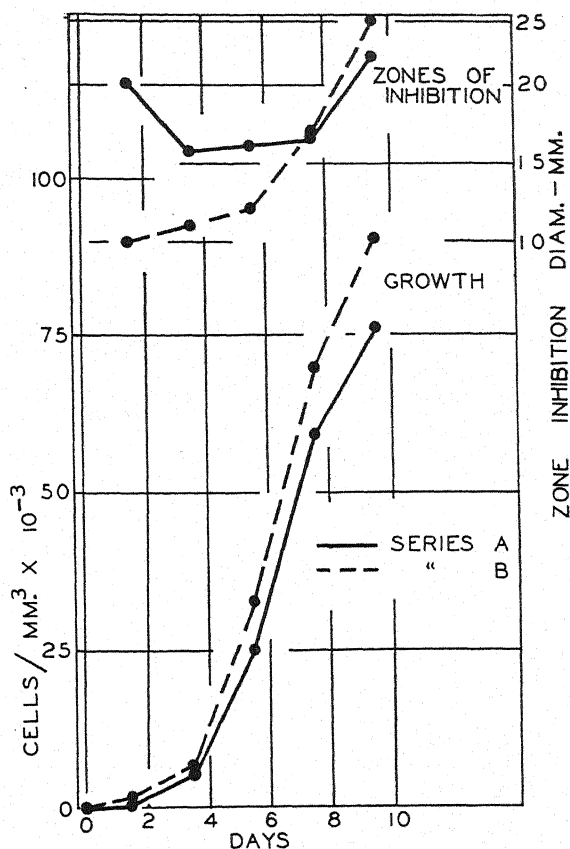


Fig. 2. (Upper portion.) Diameter of zone of inhibition caused by extracts from cultures of different ages on plates of *Staph. aureus*. A value of 8 mm. indicates no zone of inhibition, since that is the diameter of the standard cylinder. (Lower portion.) Population in *Chlorella vulgaris* cultures of different ages. See text for explanation of two series.

of dividing cells per thousand cells present is a constantly decreasing function of the age of the culture (Pratt, 1940). Hence, as time progresses, the age of the "average" cell must continuously increase, at least until sometime after the maximum population has been attained. Therefore, if the phenomenon reported by Maximov and Mozhaeva should be found to apply to the unicellular cryptogams as well as to the cells of phanerogams, it might assume major importance in the final elucidation of the mechanisms that are responsible for the changes in the concentration of chlorellin that occur in aging cultures of *Chlorella*.

SUMMARY

Cultures of *Chlorella vulgaris* were grown in continuous light in an inorganic nutrient solution enriched by a mixture of 5 per cent CO₂ in air. At intervals over a period of about two months the cells were removed from different cultures, and extracts prepared from the cell-free solutions were tested by the cylinder plate method for antibiotic activity against *Staph. aureus*.

During the thirty-six to forty-eight hours immediately following inoculation of the cultures chlorellin accumulates in the external solution. This is followed by a sharp decrease in chlorellin concentration during the next two to four days. From about the fifth or sixth day to about the fourteenth day, there is a continuous rise of chlorellin concentration which then remains relatively constant for at least six weeks.

Other experiments showed that chlorellin accu-

mulates in the culture solution when growth in population is relatively slow. During the periods of most rapid increase in density of population chlorellin disappears from the external solution. Possible interpretations of the data are discussed.

UNIVERSITY OF CALIFORNIA,
COLLEGE OF PHARMACY,
THE MEDICAL CENTER,
SAN FRANCISCO 22, CALIFORNIA

LITERATURE CITED

- ABRAHAM, E. P., ET AL. 1941. Further observations on penicillin. *Lancet* 141:177-188.
- MAXIMOV, N. A., AND L. V. MOZHAeva. 1944. Age variations in the colloid-chemical properties of protoplasm of vegetable cells. -II. Variations in permeability and viscosity in the leaf cells of broadbeans and oats. *Compt. Rend. (Dok.) Acad. Sci. U.R.S.S.* n.s. 42:277-280.
- PRATT, R. 1940. Influence of the size of the inoculum on the growth of *Chlorella vulgaris* in freshly prepared culture medium. *Amer. Jour. Bot.* 27:52-56.
- . 1942. Studies on *Chlorella vulgaris*. V. Some properties of the growth inhibitor formed by *Chlorella* cells. *Amer. Jour. Bot.* 29:142-148.
- . 1943. Studies on *Chlorella vulgaris*. VI. Retardation of photosynthesis by a growth-inhibiting substance from *Chlorella vulgaris*. *Amer. Jour. Bot.* 30:32-33.
- . 1944. Studies on *Chlorella vulgaris*. IX. Influence on growth of *Chlorella* of continuous removal of chlorellin from the culture solution. *Amer. Jour. Bot.* 31:418-421.
- , ET AL. 1944. Chlorellin, an antibacterial substance from *Chlorella*. *Science* 99:351-352.

A VIRUS TUMOR DISEASE OF PLANTS¹

L. M. Black

THE DISCOVERY of a new plant virus, *Aureogenus magnivena* Black, and data on its specific transmission by the agallian leafhoppers *Agallia constricta* Van Duzee, *Agallia quadripunctata* (Provancher) and *Agalliopsis novella* (Say) were reported in earlier papers (Black, 1943, 1944).

It is the purpose of this publication to describe the symptoms produced by this virus in a number of plants. To do this, experiments elucidating the host range of the virus will be briefly described first. Then will follow a description of the various symptoms, including tumors formed by the virus on roots of many of the susceptibles and tumors formed on the stems, petioles and leaves of certain others.

In an earlier paper (Black, 1944) the appellations "clover big-vein virus" and "clover big-vein" were suggested for the pathogen and the disease it causes in *Trifolium incarnatum* L. It now seems that the terms "wound-tumor virus" and "wound-tumor disease" may be more appropriate and distinctive and they are proposed here as alternative names preferable to those originally suggested. The reasons for choosing these names will be discussed in greater detail later.

HOST RANGE.—The host range studies reported in this paper constitute only a preliminary survey of the possible susceptibles of the virus. The species tested were selected on the basis of their availability and their adaptation to greenhouse culture. Many were ornamentals, some were vegetables and some were common weeds. The weed seeds were kindly

provided by Dr. S. G. Younkin; the seeds of cultivated plants were purchased from various seed companies. Leafhoppers of the species *Agallia constricta*, fed on diseased crimson clover for two to three weeks and then kept on healthy clover for an additional week, were used to inoculate the plants. Three experiments, each of which included most of the species, were conducted. In each experiment five insects were placed on each of two seedlings of a species and allowed to feed on the plants for a week. The insects were then removed and the plants observed in a greenhouse during a period of not less than six weeks. Some were observed for more than a year. Two uninoculated plants were retained as controls. If one or more of the inoculated plants developed symptoms typical of the disease the species was judged to be susceptible. The symptoms of wound-tumor disease were so distinctive that this procedure was considered sound, especially since any doubtful cases were omitted from the list of susceptibles presented in table 1.

The 43 susceptibles listed in the table belong to 20 families. The authorities for the names of the cultivated plants are those assigned by Bailey (1925) while the authorities for the wild species are those given by Gray (1908). Of the species listed, *Brachycome iberidifolia*, *Chrysanthemum leucanthemum* var. *pinnatifidum*, *Melilotus alba*, *Trifolium incarnatum*, *Rumex acetosa*, *Linaria maroccana*, and *Schizanthus wisetonensis* appeared to be especially easy to infect by means of insects. It is evident that the virus has a wide host range. As the three tests

¹ Received for publication April 9, 1945.

were by no means exhaustive the results indicate that the virus probably is able to infect many other genera and species in the families listed and also may be able to infect species in many additional families.

SYMPTOMATOLOGY.—The most common symptoms of wound-tumor disease are vein-enlargement and the occurrence of woody tumors on the roots of in-

TABLE 1. *Plants susceptible to wound-tumor virus.*

Family	Genus and species
Aizoaceae	<i>Tetragonia expansa</i> Murr.
Apocynaceae	<i>Vinca rosea</i> L.
Boraginaceae	<i>Heliotropium peruvianum</i> L.
Caryophyllaceae	<i>Dianthus armeria</i> L.
Caryophyllaceae	<i>Dianthus barbatus</i> L.
Caryophyllaceae	<i>Lychnis alba</i> Mill.
Caryophyllaceae	<i>Silene latifolia</i> (Mill.) Britten and Rendle
Chenopodiaceae	<i>Kochia scoparia</i> Schrad. var. <i>trichophila</i> Bailey
Compositae	<i>Anthemis cotula</i> L.
Compositae	<i>Brachycome iberidifolia</i> Benth.
Compositae	<i>Chrysanthemum leucanthemum</i> L. var. <i>pinnatifidum</i> Lecoq. and Lamotte
Compositae	<i>Rudbeckia bicolor</i> Nutt.
Compositae	<i>Rudbeckia hirta</i> L.
Compositae	<i>Venidium fastuosum</i> Stapf
Cruciferae	<i>Barbarea vulgaris</i> R. Br.
Cruciferae	<i>Capsella bursa-pastoris</i> (L.) Medic.
Cruciferae	<i>Heliophila linearifolia</i> Burch.
Cruciferae	<i>Lepidium campestre</i> (L.) R. Br.
Cruciferae	<i>Lepidium virginicum</i> L.
Cruciferae	<i>Matthiola bicornis</i> DC.
Dipsaceae	<i>Scabiosa atropurpurea</i> L.
Hydrophyllaceae	<i>Phacelia campanularia</i> Gray
Leguminosae	<i>Melilotus alba</i> Desr.
Leguminosae	<i>Trifolium incarnatum</i> L.
Leguminosae	<i>Trifolium pratense</i> L.
Linaceae	<i>Linum grandiflorum</i> Desf.
Lobeliaceae	<i>Lobelia erinus</i> L.
Nolanaceae	<i>Nolana atriplicifolia</i> D. Don.
Polygonaceae	<i>Rheum rhaponticum</i> L.
Polygonaceae	<i>Rumex acetosa</i> L.
Polygonaceae	<i>Rumex acetosella</i> L.
Polygonaceae	<i>Rumex crispus</i> L.
Polygonaceae	<i>Rumex obtusifolius</i> L.
Portulacaceae	<i>Portulaca grandiflora</i> Hook.
Portulacaceae	<i>Portulaca oleracea</i> L.
Primulaceae	<i>Anagallis linifolia</i> L.
Resedaceae	<i>Reseda odorata</i> L.
Scrophulariaceae	<i>Collinsia bicolor</i> Benth.
Scrophulariaceae	<i>Linaria maroccana</i> Hook.
Scrophulariaceae	<i>Torenia fournieri</i> Lind.
Solanaceae	<i>Nierembergia frutescens</i> Dur.
Solanaceae	<i>Schizanthus wisetonensis</i> Low.
Verbenaceae	<i>Verbena hybrida</i> Voss.

fected plants. This is emphasized so that the importance of these symptoms will not be lost sight of in the detailed symptomatological picture that follows.

Symptoms on the tops.—Most virus diseases of plants first betray their presence by the well known symptom "clearing of the veins." The wound-tumor disease is an exception to this rule. Clearing of the

veins has never been observed in any of its susceptibles. Instead an irregular enlargement of the veins is an almost universal symptom. The enlargement occurs on the lower surface of the leaf, and on the upper surface is frequently accompanied by the depression of the vein below the upper surface of the lamina. In many species the vein enlargement gives rise to growths, some of which are perhaps best described as enations, as in *Vinca rosea* and *Trifolium incarnatum*. In *Lobelia erinus* (fig. 1) large enations may be produced and small nodular growths may cluster around their base. Irregularly enlarged veins with various protuberances are illustrated by the growths on infected leaves of *Rumex acetosa* (fig. 2a and 2b). In still others the outgrowths might properly be termed vein-tumors, as in some old diseased leaves of *Rumex acetosa* or *Chrysanthemum leucanthemum* var. *pinnatifidum*. Such vein-tumors break through the overlying green tissues exposing the white tumor tissue. The overgrowths are always on the under sides of the leaves. In some species, such as *Silene latifolia* and *Rumex obtusifolius*, symptoms on the veins are so slight and so scarce that they are difficult to detect, even though upon examination the roots show numerous tumors.

It is common for diseased leaves to show various kinds of distortion, dwarfing, and curling. The tops of diseased plants are frequently severely stunted, the leaves being small and the internodes shortened. A rosette of diseased stems and leaves results (fig. 3). The crowns of infected *Chrysanthemum leucanthemum* plants may show overgrowths which appear to develop at the points where, in healthy plants, adventitious roots arise (fig. 5). In some individuals of *Brachycome iberidifolia* the stem itself is greatly thickened. Sometimes small tumors develop on petioles of infected *Rumex acetosa*. These may break through the green epidermis and expose the white tumor tissue. The production of flowers is frequently suppressed.

The species *Melilotus alba* deserves special mention because infected plants of this species produce numerous tumors on both stems and roots. The stem-tumors may attain a diameter of about 1 cm. Some are illustrated in figure 6, as are the root-tumors that have pushed their way above the surface of the soil. With the exception of overgrowths on stems at the crowns of plants, *Melilotus alba* is the only plant thus far tested that does produce tumors on the stems. It is rather surprising that it is also one of the very few that shows no symptoms on the leaves other than a slight dwarfing effect.

Some infected plants with apparently normal tops may have marked root-tumors. *Portulaca oleracea* and *Reseda odorata* are in this category. Among the small number of infected plants of these species grown in pots in the greenhouse no top symptoms, not even a reduction in growth, was observed. However, it seems probable that comparisons between groups of diseased and healthy plants grown under ideal conditions would reveal some degree of dwarfing of the tops of the diseased individuals.

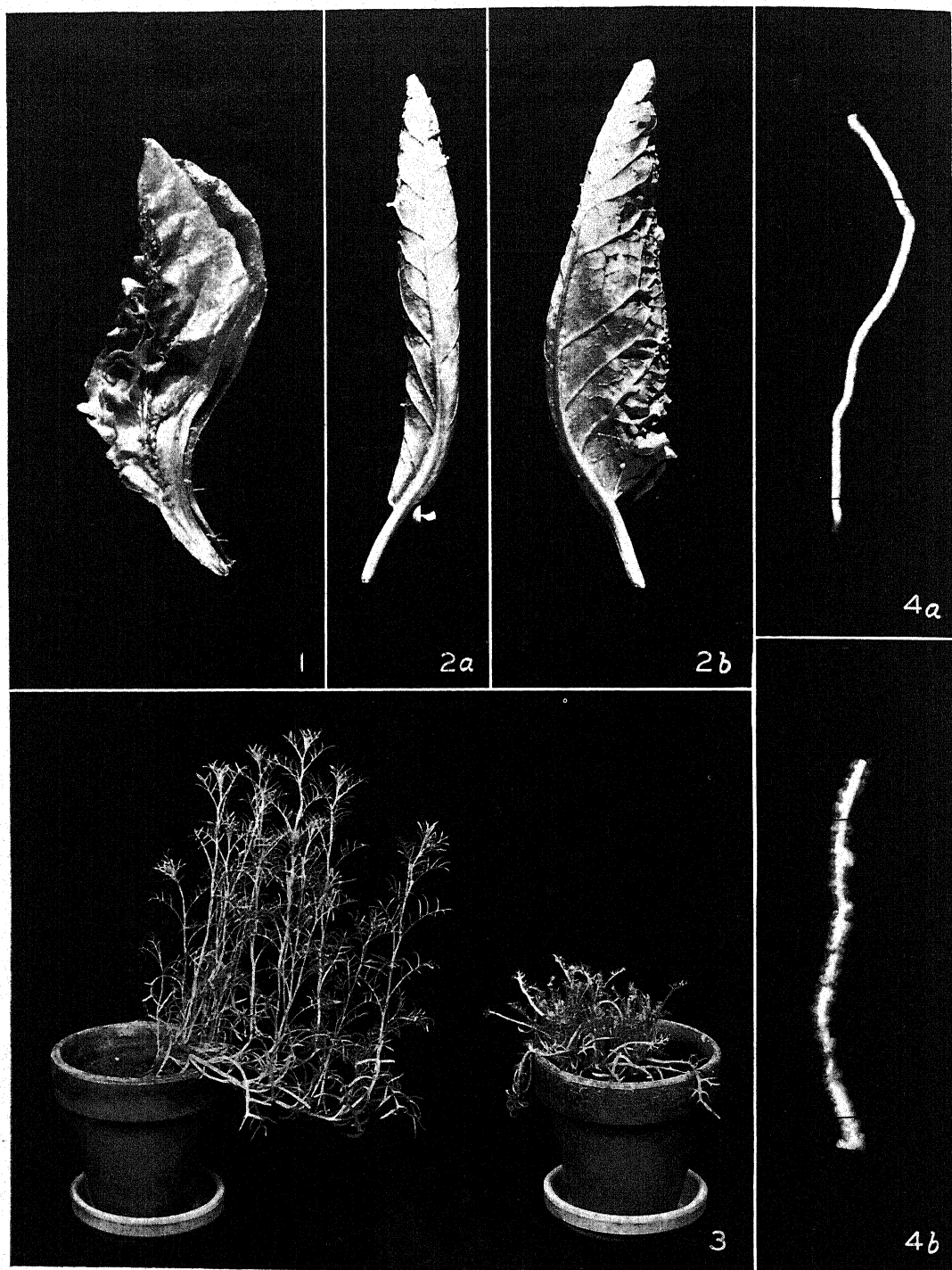


Fig. 1-4.—Fig. 1. Diseased leaf of *Lobelia erinus*. The two halves of the leaf are curled up from the midrib. From the lower side of the midrib numerous leafy enations have arisen and small nodular growths cluster about their base. ($\times 2.6$).—Fig. 2a and 2b. Diseased leaves of *Rumex acetosa* showing enlarged veins and vein protuberances of various shapes. ($\times 0.74$).—Fig. 3. *Brachycome iberidifolia*. Left: healthy; right: diseased. The internodes of the infected plant are severely dwarfed so that a rosette is formed. ($\times 0.23$).—Fig. 4a and 4b. Portions of the roots of *Reseda odorata*, a: healthy; b: diseased. On the diseased root numerous tumors have fused together to form two rows directly opposite each other. This tumor pattern corresponds to the pattern of emergence of the lateral roots, all of which were removed in preparing the specimens for photography. ($\times 0.71$). (Photographs by J. A. Carlile.)

In some species there is considerable variation in the response of different individuals to infection with the virus. In *Rumex acetosa* some individuals have very mild symptoms on the veins while other individuals produce numerous overgrowths on veins that are enlarged throughout all the leaves. There is also a marked variation in the number and size of the root-tumors on different individuals of *R. acetosa*. In *Melilotus alba* the number of tumors produced on the stems of different infected plants is subject to much variation. Moreover, the character of the tumors on different plants may vary. On most the tumors are smooth and round, but on others they may be flat, warty and elongated along the stem.

Symptoms on the roots.—The virus produces tumors on the roots of many different kinds of plants. On some plants such as *Kochia scoparia* var. *trichophila* or *Heliotropium peruvianum* only one or a few tumors may be produced on an extensive root system. These may be clustered as observed in an infected *Kochia* root or scattered as for example in *heliotrope*. On the other hand in *Portulaca oleracea*, *Lepidium campestre*, *Melilotus alba* and others the root-tumors may be exceedingly numerous. In some species the tumors may be so close together that they are fused. Figures 4a and 4b illustrate these features in roots of *Reseda odorata*. There are some susceptibles of the virus which do not produce noticeable root-tumors when infected. For example, infected plants of *Lobelia erinus*, although they have exhibited striking leaf symptoms, have shown no macroscopic tumors on the roots. However, even in this case very small swellings on the vascular system of the root are detectable through the transparent cortex by means of the naked eye.

In a number of species the tumors obviously originate at the points where the lateral roots emerge from the main root and it may well be that such a relationship holds true for most or all tumors on the roots of other susceptibles. For example, in *Reseda odorata* the lateral roots emerge for the most part in two rows on opposite sides of the mother root. The tumors on infected roots of this species have the same distribution (fig. 4a and 4b) and so give the diseased root an undulating, ribbon-like appearance. In *Dianthus barbatus* the tumefactions appear as collar-like growths around the base of the lateral roots (fig. 7). It may be that the wound produced by the emergence of the lateral root plays a part in the origin of the tumor at that point.

In size the root-tumors range from mere pustules on the fine roots of some cruciferous species and other plants to tumors measuring about 1 cm. in diameter on the roots of certain infected individuals of *Rumex acetosa* (fig. 9a and 9b). The root-tumors of *Silene latifolia* (fig. 8) and of *Melilotus alba* (fig. 6) are also relatively large. In *Rumex acetosa* and *Melilotus alba* roots having a diameter of 0.3 mm. may bear spherical tumors with a diameter ten times as great. However, the tumors growing on the susceptibles are limited in size. This limit varies from one species to another and is probably determined by

the failure of the disorganized vascular system of the tumors to provide adequate food transport over more than a short distance.

On all plants the tumors seem to have a woody texture and a more or less spherical structure that permits distinction from the commonly fusiform parenchymatous galls caused by the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. The tumors are also readily distinguished from the nodules on legume roots caused by species of *Rhizobium* even when both occur on the same root. The bacterial nodules are more elongated, smoother, softer, more regular in outline and are much more fragile.

On most roots the tumors are the same color as the roots on which they are borne but on the roots of *Anagallis linifolia* they contain varying amounts of a purple pigment. Sometimes these tumors are dark purple, which is in striking contrast to the light color of the normal root. In this connection it should be mentioned that normal plants of this species produce warty overgrowths on the stems and that these also contain a purple pigment.

Old root tumors rot, probably because of invasion by secondary organisms, so that old infected roots may show extensive decay.

DISCUSSION.—Wound-tumor disease would seem to provide excellent biological material for studying plant tumors of virus causation. That the pattern of occurrence of the tumors on certain roots is identical with the pattern of origin of the lateral roots has already been pointed out. Lateral roots originate as meristems in the pericycle and break through the endodermis, cortex and epidermis. In emerging they always wound these overlying tissues. On the other hand leaves, branches and flowers are initiated in the superficial layers of the promeristem of the stem tip and develop without breaking through and wounding pre-existing tissues. Preliminary experiments clearly indicate that wounds are involved in the origin of at least some of the tumors. It therefore seems probable that the wounds made by emerging lateral roots are connected with the high frequency of root-tumors, whereas the origin of the appendages of the stem without comparable wounds may be an important factor accounting for the relative infrequency of stem-tumors. Moreover, wounds offer an explanation for the local character of individual tumors, which require as one essential condition of their development a systemic virus. The origin of the overgrowths exclusively in the vicinity of the vascular system suggests that the virus exhibits tissue specialization in its effects, and the occurrence of the overgrowths only on the lower sides of leaves suggests that the phloem is probably the tissue affected. Cytological and tissue culture studies, although only in a preliminary stage, indicate clearly that meristems are continuously present in the tumors and that these overgrowths have potentialities for unlimited growth without differentiation into normal plant organs such as roots, stems or leaves.

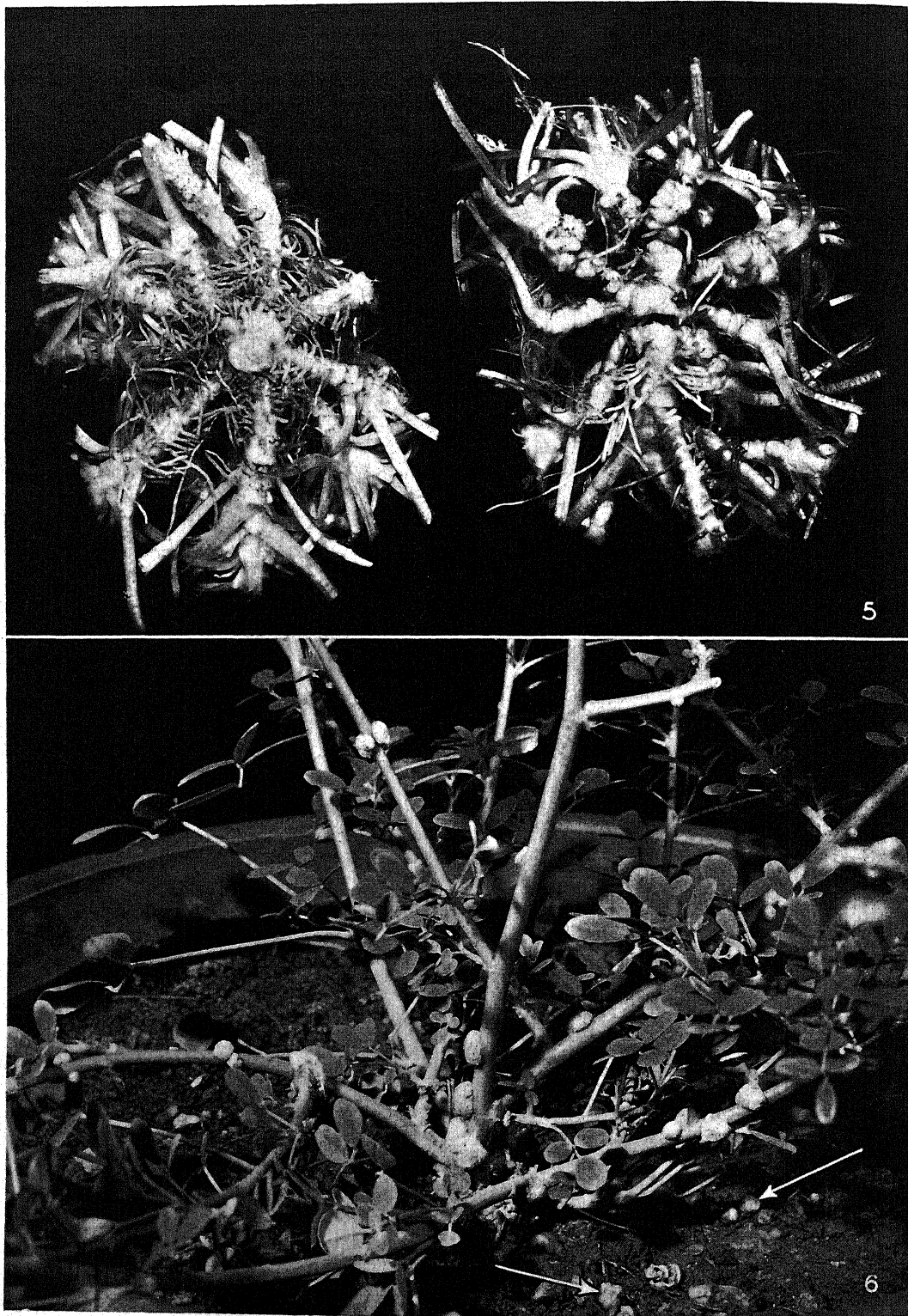


Fig. 5-6.—Fig. 5. Crowns of *Chrysanthemum leucanthemum* var. *pinnatifidum* photographed from the under side. Left: healthy; right: diseased. The diseased crown has numerous tumors. ($\times 0.91$).—Fig. 6. Diseased biennial white sweet clover plant, *Melilotus alba*. Numerous tumors may be seen on the stems, and root-tumors (indicated by arrows) that have grown above the surface of the soil are plainly visible. ($\times 0.91$). (Photographs by J. A. Carlile.)

Wound-tumor disease resembles Fiji disease of sugar cane, which occurs in New Guinea, Fiji, Java, the Philippines and Australia (Holmes, 1939). The virus causing Fiji disease is transmitted by two species of leafhoppers in the genus *Perkinsiella* (Mungomery and Bell, 1933; Ocfemia, 1934). The only known host is sugar cane, *Saccharum officinarum* L., which when infected develops galls on the veins on the under sides of the leaves and in the tissues of the stalk. These galls originate in the phloem (Kunkel, 1924). McWhorter (1922) reported the formation of galls on the roots but Ocfemia (1934) reported that he noted no changes in the roots of experimentally infected plants. The galls of Fiji disease appear to mature (Kunkel, 1924) and this may be a difference between the overgrowths of Fiji disease and those of wound-tumor disease. Wound-tumor disease may also be similar to Wallaby Ear disease of corn about which very little has thus far been published (Schindler, 1942). The possibility of relationship between the wound-tumor virus and the virus causing curly-top of beets in Argentina has already been suggested (Black, 1944). In many ways wound-tumor disease appears to be a homologue in plants of the tumor diseases in animals that are caused by viruses (Andrewes, 1934; Rous, 1943). Very little seems to be known about the natural means of spread of such animal viruses.

In regard to several of its characters wound-

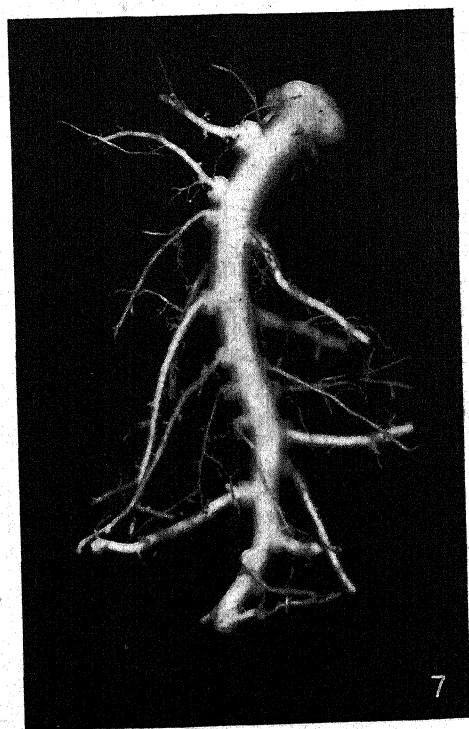


Fig. 7. Portion of a diseased root of *Dianthus barbatus*. The tumefactions appear as collar-like growths around the lateral roots where they emerge from the main root. ($\times 1.6$). (Photograph by J. A. Carlile.)

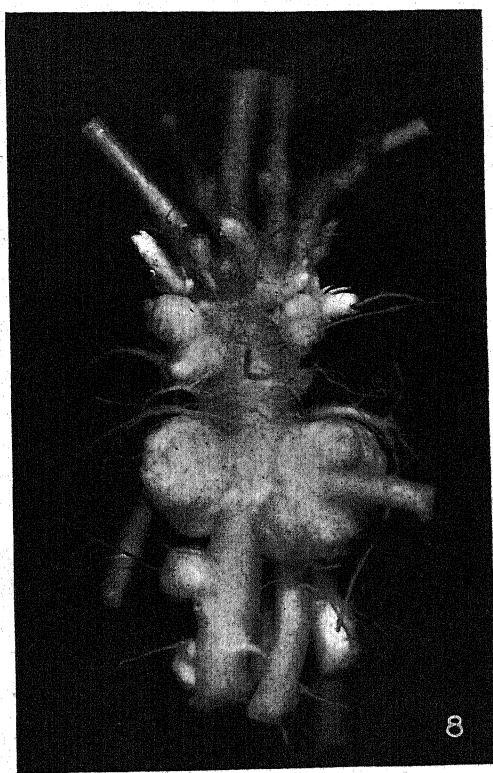


Fig. 8. Portion of a diseased root of *Silene latifolia* showing a large root-tumor. ($\times 1.6$). (Photograph by J. A. Carlile.)

tumor disease appears to resemble crown-gall in spite of the bacterial origin of the latter. It differs from crown-gall, however, not only in etiology but also in its systemic nature. For, in spite of the production of a limited number of secondary tumors in certain hosts (Smith, 1920; Braun, 1941) crown-gall is essentially a localized infection and not a generalized or systemic disease.

Although the writer is not certain that all tumors arise at the site of a wound it is certain from preliminary experiments that some of them do. The probable relationship between wounds and tumors is clearly indicated in the above discussion of symptoms in relation to plant anatomy. Hence the writer's inclusion of the word "wound" in the names for disease and virus. While the use of the word "tumor" is not without precedent in plant pathology (Smith, 1920; Braun, 1941; White, 1944) the word gall has so much wider usage that it seems incumbent on the writer to explain his choice. He has employed the term tumor rather than gall to describe the overgrowths of wound-tumor disease for two reasons. Webster (1932) defines a tumor as "an abnormal mass of tissue, *not inflammatory* and independent in character, *arising without obvious cause from cells of pre-existent tissue*, possessing no physiologic function, and characteristically unrestrained in growth and structure." A gall is defined as "a swelling or excrescence of the tissues of plants resulting from

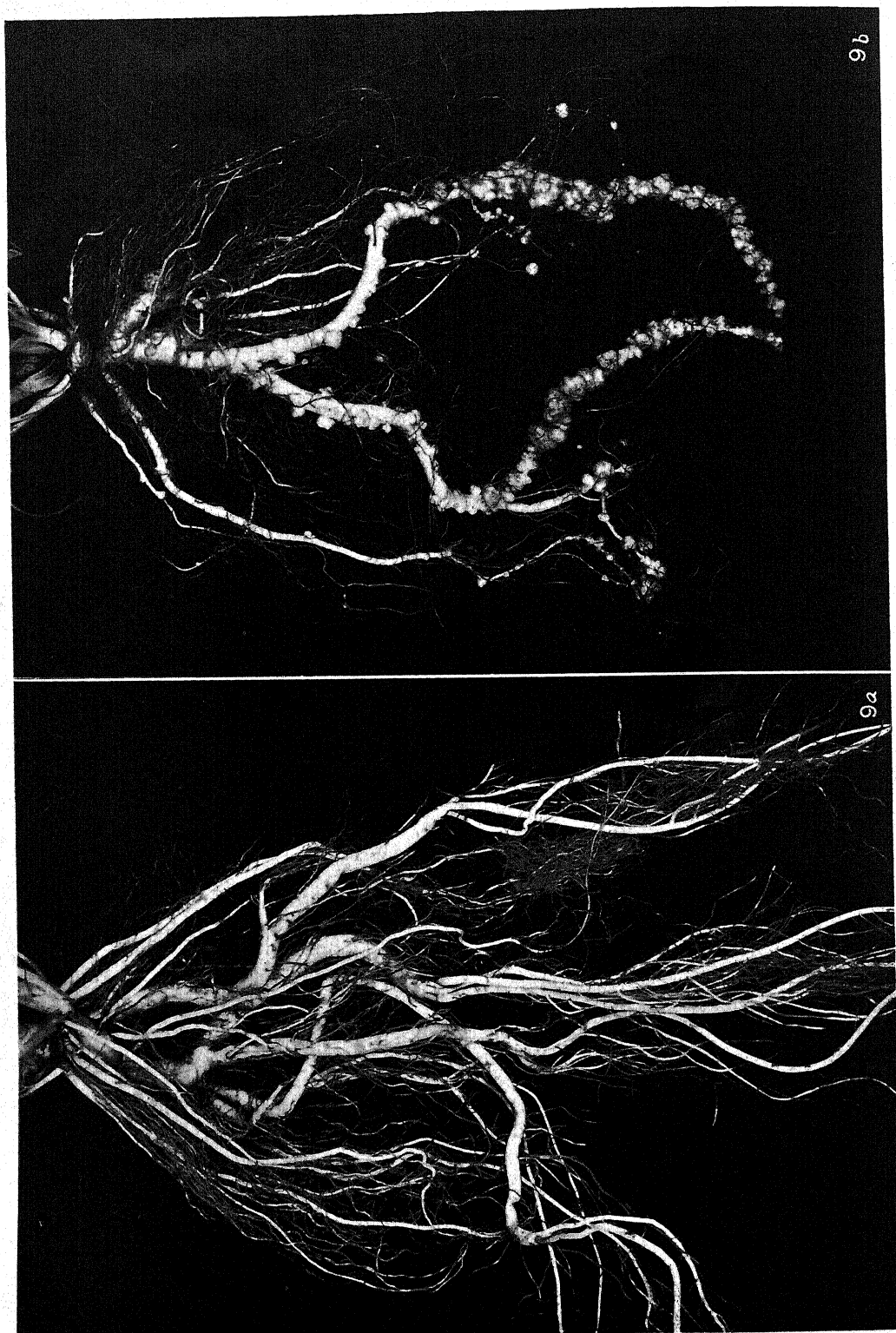


Fig. 9a and 9b. Roots of *Ramea acetosa*: a, healthy; b, diseased. ($\times 0.64$). (Photographs by J. A. Carlile.)

the attacks of certain parasites, which cause an abnormal and sometimes very extraordinary proliferation of the cells of the host plant." The italics are the writer's and if the italicized phrases are omitted, the first because of its irrelevance for plants and the second because it is purely subjective, the definition of tumor will be found to describe the characters of the overgrowths of wound-tumor disease more precisely than the definition given for a gall. The use of "tumor" also favors comparisons, which may be mutually enlightening, between animal and plant tumors, whereas the employment of the purely botanical term "gall" does not.

The production of tumors in plants infected by the virus, *Aureogenus magnivena*, raises doubts as to the generic relationship between this virus and the other viruses in *Aureogenus*. *A. magnivena* was not known to produce tumors in plants when it was named and placed in the genus. However, until more is known about the virus which will help in its classification in a natural system it seems best to leave it as a species of *Aureogenus*.

SUMMARY

The tumors produced by the virus *Aureogenus magnivena* on the roots of many susceptibles are described and illustrated. The overgrowths tended to

be spherical and woody. Depending on the species of suspect they ranged in size up to 1 cm. in diameter and were so numerous on some roots that they were fused together. On other species only a few scattered tumors arose. On still others a cluster of tumors appeared on a single lateral root, other roots on the plant appearing healthy. In one species the root-tumors were a deep purple although the normal root was almost white. There appeared to be a definite connection between the root-tumors and the emergence points of the lateral roots.

On *Melilotus alba* tumors appeared on the stems as well as on the roots. On most species the veins of the leaves were irregularly enlarged and, on many, various overgrowths such as enations or vein-tumors were formed on the under side. Tumors were also produced on the crowns of some hosts and on the petioles of others.

Forty-three species of plants in 20 families were infected by the virus and it is believed that many more susceptibles remain to be discovered.

It is suggested that the names "wound-tumor disease" and "wound-tumor virus" for the disease and for the pathogen respectively are preferable to "clover big-vein" and "clover big-vein virus."

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY

LITERATURE CITED

- ANDREWES, C. H. 1934. Viruses in relation to the aetiology of tumours. The Oliver-Sharpey Lectures. The Lancet 227: 63-69, 117-124.
- BAILEY, L. H. 1925. Manual of cultivated plants. 851 pp. Macmillan Co., New York.
- BLACK, L. M. 1943. Two new viruses transmitted by agallian leaf hoppers. (Abst.) Phytopath. 33: 1110.
- . 1944. Some viruses transmitted by agallian leaf hoppers. Proc. Amer. Philos. Soc. 88: 132-144.
- BRAUN, A. C. 1941. Development of secondary tumors and tumor strands in the crown gall of sunflowers. Phytopath. 31: 135-149.
- GRAY, A. 1908. New manual of botany. 7th edition, revised by B. L. Robinson and M. L. Fernald. 926 pp. American Book Co., New York.
- HOLMES, F. O. 1939. Handbook of phytopathogenic viruses. 221 pp. Burgess Publishing Co., Minneapolis.
- KUNKEL, L. O. 1924. Histological and cytological studies on the Fiji disease of sugar cane. Hawaiian Sugar Planters' Assoc. Exp. Sta. Bull. Bot. ser. 3(2): 99-107.
- MCWHORTER, F. P. 1922. The nature of the organism found in the Fiji galls of sugar-cane. Phil. Agric. 11: 103-111.
- MUNGOMERY, R. W., AND A. F. BELL. 1933. Fiji disease of sugar-cane and its transmission. Queensland Bur. Sugar Exp. Sta., Div. Path. Bull. 4. 1-28.
- OCFEMIA, G. O. 1934. An insect vector of the Fiji disease of sugar cane. Amer. Jour. Bot. 21: 113-120.
- ROUS, P. 1943. Viruses and tumors. IN: Virus diseases, The Messenger Lectures, pp. 147-170. Cornell Univ. Press, Ithaca.
- SCHINDLER, A. J. 1942. Insect transmission of Wallaby Ear disease of maize. Jour. Australian Inst. Agric. Sci. 8: 35-37.
- SMITH, E. F. 1920. The crown gall. IN: An introduction to bacterial diseases of plants. pp. 413-472. Saunders Co., Philadelphia.
- WEBSTER, N. 1932. Webster's new international dictionary of the English language. Edited by W. T. Harris and F. S. Allen. 2620 pp. Merriam Co., Springfield, Mass.
- WHITE, P. R. 1944. Transplantation of plant tumors of genetic origin. Cancer Research 4: 791-794.

AN EXPERIMENTAL ANALYSIS OF ALKALOID PRODUCTION IN NICOTIANA: THE ORIGIN OF NORNICOTINE ¹

Ray F. Dawson

THE NICOTIANA alkaloids constitute a remarkable natural series the common denominator in which is the presence of a pyridine nucleus. Such similarity in basic molecular structure would seem to bespeak a degree of unity in the origins of these interesting compounds, which unity might, in turn, conceivably provide a key to the experimental elucidation of their chemical synthetic mechanisms. The present communication provides an instance of such a relationship by demonstrating that nornicotine (I) is produced *in vivo* from nicotine (II) probably by a process of transmethylation. It is furthermore indicated that the inheritance of this mechanism accounts for the predominance of nornicotine over nicotine in hybrids between *Nicotiana tabacum* and *N. glutinosa* and for the alleged predominance of anabasine (III) (really the predominance in the genetical sense of nornicotine) over nicotine in hybrids of the type *N. tabacum* \times *N. glauca* (cf. Smith and Smith, 1942).

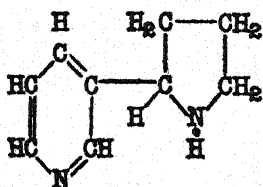
The experimental approach to the solution of these problems was necessarily based upon the possession of complete information concerning the loci of alkaloid synthesis within the plant bodies of those species which produce nicotine, nornicotine, and anabasine, respectively. Nicotine has already been shown to originate solely in the roots of *N. tabacum* and of *N. glauca* (Dawson, 1942, 1944). Anabasine, on the other hand, is apparently formed in both root and shoot of *N. glauca* (Shmuck, Smirnov and Ilyin, 1941; Dawson, 1944). The localization of the synthetic mechanism of the third and last of the major *Nicotiana* alkaloids, nornicotine, in *N. glutinosa* and in *N. glauca* has therefore been investigated and is reported in this paper.

and of transformation in the three *Nicotiana* species, *tabacum*, *glauca*, and *glutinosa* and in the hybrid *N. tabacum* \times *N. glauca*. The experimental basis upon which these interpretations rest is presented in the following sections.

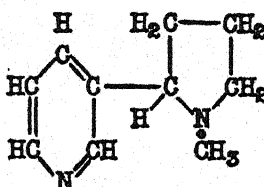
METHODS.—Reciprocal grafts involving tomato (vars. Rutgers and Bonny Best), *Nicotiana glutinosa* (seed obtained through the courtesy of the Office of Tobacco and Plant Nutrition of the Bureau of Plant Industry), *N. glauca*, and *N. tabacum* var. Turkish were prepared according to procedures described elsewhere (Dawson, 1942, 1944a). The hybrid *N. tabacum* \varnothing \times *N. glauca* δ was obtained from the same strains that were used in the grafting experiments. In all cases graft unions were located as near to the ground as possible without incurring the danger of formation of adventitious roots by the scions. The stocks were always defoliated unless otherwise indicated.

The grafted plants were grown in an outdoor plot during the summer of 1944. The hybrids were grown in the greenhouse during the winter of 1944–1945. These were harvested and prepared for analysis according to the usual procedures employed in this laboratory (Dawson, 1942, 1944).

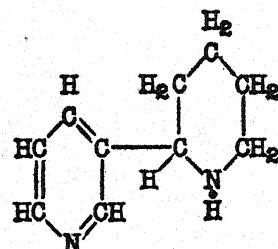
In all cases the alkaloids were initially separated from the plant material by steam distillation from strongly alkaline, saturated sodium chloride solution into dilute hydrochloric acid. Distillation was continued until 10 milliliters of the distillate when treated with a few drops of silicotungstic acid solution, heated, then cooled in running tap water for five minutes, yielded no opalescence. It should be noted that such tests for alkaloids in the distillates will remain positive long after an opalescence has



(I)



(II)



(III)

Finally, through the use of suitable graft combinations, it has been possible to chart in a general way not only the points of origin of each of these three alkaloids but also the centers of accumulation

¹ Received for publication April 5, 1945.

The author wishes to express his indebtedness to the Rockefeller Foundation for financial assistance and to Professor K. W. Cooper and Doctor W. H. Pearlman for their many generous contributions of ideas during the course of this investigation.

ceased to form in parallel tests conducted throughout at room temperature. Much nornicotine may be lost if this precaution is not observed.

After concentration to low volume under reduced pressure the distillates were made alkaline and distilled slowly through a six-inch Widmer column into dilute hydrochloric acid to remove nicotine and the lower boiling bases (Smith and Smith, 1942). The alkaline solutions of non-volatile alkaloids remain-

ing from these distillations were then chilled, saturated with solid sodium chloride, filtered, and extracted with ether. The ether extracts were freed from the ether by distillation and the alkaloids taken up in hot aqueous picric acid solution from which the picrates crystallized on cooling. The distillates from the Widmer column were evaporated to dryness under reduced pressure, a little water added and the process repeated to remove the last traces of hydrogen chloride. The resulting white crystalline material, usually very hygroscopic, was taken up in a small volume of water and picric acid solution added as before. The yields of the various samples are based upon the air dry weights of these crude alkaloid picrates.

The isolation of individual alkaloids from these crude preparations proceeded as follows. In nearly all cases, one recrystallization of the picrates of the alkaloids that distilled through the Widmer column was sufficient to yield pure nicotine dipicrate. The isolation of individual alkaloids from the non-volatile fractions was considerably more difficult in those cases where more than one alkaloid occurred in appreciable amount. In these instances two procedures were followed. First a careful fractional crystallization of the picrates was carried out with close attention to the melting point of each fraction. Secondly, if mixtures of nornicotine and anabasine were suspected, portions of the mixed picrates were methylated *in toto* with the aid of formaldehyde and formic acid (Bowen and Barthel, 1944a), and the methylated picrates subjected to repeated fractional crystallization with emphasis upon the least-soluble nicotine-rich fractions.

In carrying out such fractional crystallizations three difficulties were frequently encountered. First, in some samples there occurred a steam-volatile substance which did not distill through the Widmer column and which gave a troublesome oily picrate the presence of small amounts of which sufficed to depress strongly the melting points of anabasine or nornicotine dipicrates. Fortunately, this substance could be largely eliminated by filtering the hot solutions of the alkaloid picrates through cotton wool during the first recrystallization. Secondly, the work of Bowen and Barthel (1944) and the data reported in this paper upon the physical properties of synthetic mixtures of the alkaloid picrates make it clear that eutectic mixtures between any two can be anticipated. Furthermore, it is evident (cf. figure 1) that the properties of these eutectic mixtures may be quite different from the properties of either alkaloid alone. It should be mentioned in this connection that fractional crystallizations performed upon crude picrates containing nicotine (cf. Vickery and Pucher, 1930) almost certainly result in the isolation of eutectic mixtures of hopelessly complex composition. While the problem of avoiding or detecting these mixtures has not been fully solved, the removal of nicotine prior to carrying out the fractional crystallization reported here-

in has greatly simplified the task of isolation and identification. Finally, the third difficulty encountered in the separation of anabasine from nornicotine was the presence in relatively small amounts in grafts involving *N. glutinosa* and *N. glauca* of at least two alkaloids of unknown identity one of which seemed to be the N-methyl derivative of the other. The picrate of one melted only a few degrees below the melting point of nornicotine dipicrate,

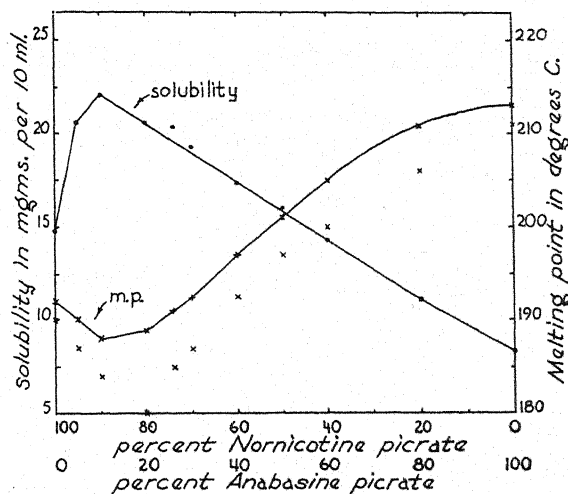


Fig. 1. Solubility and melting point spread of synthetic mixtures of anabasine and nornicotine dipicrates. Since the upper limits of the melting point spreads are more readily duplicated, they have been connected with a line to facilitate comparisons. The lower limits of the spreads represent the temperatures at which the first signs of sintering were observed in the melting point tubes. The figure, therefore, represents a nomogram which can be used to determine the composition of mixed picrates by drawing vertical lines through experimentally obtained points on the solubility and melting point curves and extending them to the scale on the abscissa. Corresponding to the respective melting points, the solubility data deviated from the theoretical by + 0.1, - 0.1, and - 0.9 mgm. for samples of picrates from *N. glauca* scions on *N. tabacum* stocks. The corresponding deviation for the mixed picrates obtained from the hybrid *N. tabacum* × *N. glauca* was + 0.6 mgm.

and the picrate of the other melted at 217–219.5°C., just a few degrees below the melting point of nicotine dipicrate. These substances always appeared in the form of very tiny crystals and sometimes occurred in sufficient amounts to necessitate carrying the picrates of nornicotine and anabasine through several recrystallizations to be rid of them.

Nicotine was identified by its azeotropic property with water at the boiling temperature, by its stability to nitrous acid, and by the melting point of its picrate (dec. 224–226°C.). Anabasine was identified by its failure to distill through the Widmer column, by the melting point of the picrate (213°C.), and by the solubility of the latter under

the conditions specified below. The identity of nornicotine was established by the same general criteria as those used for anabasine in addition to its methylation to nicotine. Since no method was available for the quantitative determination of anabasine in the presence of nornicotine a procedure was developed which gave both qualitative evidence for the occurrence of the two alkaloids in mixtures and evidence for the approximate relative proportions in which they occurred. For this purpose synthetic mixtures containing various known proportions of purified nornicotine dipicrate (m.p. 190–192°C.; picric acid content found 75.6 per cent, theory 75.5 per cent) and anabasine dipicrate (m.p. 211–213°C.; picric acid content found 73.95 per cent, theory 73.86 per cent) were prepared in 50 milligram quantities. Each mixture was dissolved in boiling water, the volume adjusted to 10 milliliters, and the tube containing the solution allowed to cool slowly at room temperature (about 25°C.). Crystallization was induced by scratching the walls of the tube with a glass stirring rod at the first appearance of a yellow cloudiness. Failure to bring about early crystallization led to greatly different results due probably to the failure of formation of maximum amounts of the eutectic mixture.² The crystalline products were then quantitatively filtered, dried at 50°C. for several hours, and weighed to the nearest tenth of a milligram. The entire melting point spread of each crystalline sample was obtained and this and the solubility plotted against the proportions of the two alkaloids in the original samples. The results are shown graphically in figure 1. Unknowns, from aliquots of which both anabasine and nornicotine had been isolated earlier, were then dissolved in the ratio of 50 milligrams of picrate to 10 milliliters of hot water and the solubilities and melting points observed. These operations were repeated a second or even third time when this became necessary to remove the last traces of contaminating alkaloids. The limitations of this arbitrary procedure are obviously (a) the

² Among the three major tobacco alkaloids the property of separating from aqueous solution during cooling as a second liquid phase is peculiar to nornicotine picrate. Transition to the solid state usually occurs in a few minutes to a few hours depending upon the purity of the preparation. If the mixed picrates of anabasine and nornicotine are precipitated rapidly in liquid form by plunging the tubes into cold water, the eutectic point disappears on the solubility curve (fig. 1) and the whole is replaced by a broad parabola. Cooling in the air at room temperature and without scratching, on the other hand, results in a broken type of solubility curve which differs from that in figure 1 only by the possession of a negative slope to the left of the eutectic point. The melting point curve to the left of the eutectic point under these conditions also shows a lower vertical displacement and then becomes very similar to the curve obtained by Bowen and Barthel (1944) for mixtures of nicotine and nornicotine dipicrates. While these last authors do not state the conditions under which crystallization occurred in their experiments, it is assumed that no effort was made to prevent the separation of liquid nornicotine dipicrate, and that, therefore, their results are comparable with those obtained in the present investigation from this standpoint.

necessity for the presence in the final analysis of only anabasine and nornicotine dipicrates and (b) the failure to indicate the true ratio between these two alkaloids in the original crude isolate.

Further manipulative details are given in connection with the experiments in which they were employed, since such information possesses a direct bearing upon the principal thesis of this paper.

The occurrence of unidentified alkaloids in individual experiments is not given consideration in this paper. The properties, origins and identities of these substances are to receive future investigation in this laboratory. There is no concrete evidence to show that they were in any direct way linked with the phenomena that are described herein.

All melting points recorded in this paper have been corrected for calibration errors and stem exposure.

RESULTS.—*Alkaloids in reciprocal grafts of Nicotiana glutinosa with N. tabacum and tomato.*—When *N. glutinosa* scions were grown on tomato stocks neither scion nor stock contained any of the three major tobacco alkaloids (table 1). On the other hand, tomato scions grown on *N. glutinosa* stocks unexpectedly contained large amounts of nicotine. This observation was confirmed by a similar experiment in which the non-alkaloid producing shoots of *N. tabacum* were grafted to *N. glutinosa* roots. Again, only nicotine could be detected in the scions. Furthermore, when tomato and *N. glutinosa* shoots were both grown on the same *N. glutinosa* roots the *N. glutinosa* shoots contained much nornicotine with only a little nicotine, while the tomato shoots contained only nicotine.

The most obvious interpretation of these observations would seem to be that nicotine is formed in the roots of *N. glutinosa*, then translocated to the shoot of the intact plant where some mechanism is present which converts it to nornicotine. The theoretical basis for such an interpretation of the results of grafting experiments has been presented in some detail elsewhere (Dawson, 1942, 1944; Peacock, Leyerle and Dawson, 1944).

If the present interpretation is correct, however, it should be possible to interchange root systems of *N. glutinosa* and *N. tabacum* without resultant qualitative differences in the alkaloid composition of the respective shoots. Such, indeed, is the case, for, as was pointed out above, only nicotine occurred in the graft type *N. tabacum* scion on *N. glutinosa* stock, while both nornicotine (dipicrate m.p. 190–192°C.; picric acid content found 75.6 per cent, theory 75.5 per cent) and nicotine occurred in the reciprocal combination (table 1). In like manner, when both *N. tabacum* and *N. glutinosa* shoots were grown on the same *N. tabacum* root, the *N. tabacum* shoot contained only nicotine, while the *N. glutinosa* shoot contained both nicotine and nornicotine. The root systems of both species are similar, therefore, in that each may be regarded essentially as a nicotine donor. The sole distinction between them from the point of view of major alkaloid genesis

is the fact that morphologically the root system of *N. tabacum* is larger and more extensive than that of *N. glutinosa* and therefore might be expected to donate more nicotine to the aerial shoot which it serves. This is borne out by a comparison of the data representing the relative contents of nicotine and nornicotine in this type of reciprocal graft in table 1.

vented or nicotine is converted into some other compound once it reaches the shoot of the intact plant of *N. glauca*. In an attempt to determine the nature of this interaction, *N. glauca* shoots were grafted to *N. tabacum* roots in the hope that the potentially augmented supply of nicotine from the *N. tabacum* donor might be reflected in a greater accumulation within the stock or scion of either the end product

TABLE 1. Alkaloid pierates isolated from various graft combinations and hybrids.

Description of sample	Number of plants sampled	Dry weight of sample	Pierates obtained ^c		
			Nicotine	Nor-nicotine	Anabasine
		g.	mg.	mg.	mg.
Tomato on <i>N. glutinosa</i> (scions).....	4	122.5 ^a	150	0	0
	4	50	358	0	0
<i>N. tabacum</i> on <i>N. glutinosa</i> (scions).....	3	550 ^a	1258	0	0
<i>N. glutinosa</i> on tomato (scions).....	3	340 ^a	0	0	0
(scions).....	2	50	0	0	0
(stocks).....	2	...	0	0	0
<i>N. glutinosa</i> on <i>N. tabacum</i> (scions).....	2	374 ^a	1092	246	0
	3	50	492	1128	0
<i>N. glutinosa</i> + <i>N. tabacum</i> on <i>N. tabacum</i>	3
<i>N. tabacum</i> scions	50	719	0	0
<i>N. glutinosa</i> scions	50	627	1218	0
<i>N. glutinosa</i> + tomato on <i>N. glutinosa</i>	1
Tomato scions	10.5	84	0	0
<i>N. glutinosa</i> scions	52	88	1439	0
Intact <i>N. glutinosa</i> leaves.....	1	52.3	160	851	0
<i>N. glauca</i> on tomato (stocks).....	2	387 ^a	0	0	0
<i>N. tabacum</i> on <i>N. glauca</i> (scions).....	5	50	28	0	50
<i>N. glauca</i> on <i>N. tabacum</i> (scions).....	3	100	0	473 ^b	577 ^b
(scions).....	1	50	29	1075 ^b	880 ^b
(stocks).....	1	225 ^a	1185	0	0
Tomato on <i>N. glauca</i> on <i>N. tabacum</i>	2
Tomato scions	9.5	61	0	0
<i>N. glauca</i> scions	50	0	347 ^b	891 ^b
Tomato on leafless <i>N. glauca</i> on <i>N. tabacum</i>
Tomato scions	2	18	28	0	0
Intact <i>N. tabacum</i> roots.....	3	...	774	0	0
<i>N. tabacum</i> × <i>N. glauca</i> (shoots).....	12	50	93	176 ^b	244 ^b
(excised root cultures).....	78	...	57	0	9
Tomato on <i>N. tabacum</i> × <i>N. glauca</i> (scions).....	5	50	479	0	443
<i>N. tabacum</i> × <i>N. glauca</i> on tomato (scions).....	2	24.5	0	0	9
<i>N. tabacum</i> × <i>N. glauca</i> on <i>N. tabacum</i> (scions).....	2	23	62	371 ^b	8 ^b

^a Fresh weight of sample.

^b Estimated amounts using the solubility-melting point method described herein. These figures are intended to give only relative approximations of the actual amounts of the two alkaloids that were present in the original samples.

^c Data on the occurrence of unidentified alkaloids are not included.

Reciprocal grafts of Nicotiana glauca with N. tabacum.—It has been shown earlier that *N. glauca* roots produce a little nicotine (Dawson, 1944). These observations have been confirmed in the present study by the isolation of both nicotine and anabasine from *N. tabacum* scions grown on *N. glauca* roots (table 1). This may be regarded as an observation of unusual interest, since nicotine has not been encountered in intact plants of the strain of *N. glauca* that is used in this laboratory. The suggestion is apparent that some type of interaction between alkaloid synthetic mechanisms occurs whereby either nicotine formation in the root is pre-

vented or the diversionary product of nicotine metabolism. According to Shmuck, Kostoff and Borozdina (1939), Shmuck (1940), and Kuzmenko and Tikhvinskaya (1940) this product should be anabasine.

From 100 grams of dried *N. glauca* scions 0.5851 gram of a yellow oily liquid was obtained which gave a yellowish opalescent suspension when placed in water. The aqueous suspension was reextracted with ether, the extract dried over anhydrous sodium sulphate, and the ether removed by distillation. The recovered alkaloid was then distilled *in vacuo* and collected as a water white condensate in the second bulb of a microsublimation tube. This

product dissolved completely in water to give a clear solution. The solution of the free alkaloids was laevorotatory with a specific rotation $[\alpha]_D$ of -21.3° . Picric acid was added to one portion of the aqueous solution and from the resulting crystalline product both nornicotine (dipicrate m.p. $184.5-186^\circ\text{C}.$ ³) and anabasine (impure dipicrate after five recrystallizations melted at $204-205^\circ\text{C}.$ ⁴) were obtained. The ratio of nornicotine to anabasine picrate in this sample as determined by the solubility-melting point method was approximately 45 to 55. When treated with nitrous acid a subsample of the mixed picrates was converted to the corresponding non-steam-volatile nitroso compounds. A third subsample when methylated with formic acid and formaldehyde yielded nicotine dipicrate (m.p. $223-224^\circ\text{C}.$, dec.) after two recrystallizations.

The above data strongly suggest that the predominance of anabasine over nicotine in grafts of this type as reported by the Russian investigators (loc. cit.) is in reality a case of normal synthesis of their characteristic alkaloids by both stock and scion followed by a secondary conversion of nicotine to nornicotine in the leaves of the scion. Indeed, these investigators did not employ methods which were capable of differentiating between anabasine and nornicotine, nor did they apparently have reason to suspect the presence of the latter alkaloid.

The above interpretation is, of course, subject to experimental test. If it is true that in such graft combinations nicotine is formed normally in the stock prior to its upward translocation, then an examination of these stocks should reveal the presence of only nicotine. Such was found to be the case (table 1). Furthermore, if the *N. glauca* scion of this graft hybrid were to be in turn surmounted by a tomato scion, the latter should contain nothing but nicotine, while the underlying *N. glauca* tissues should contain principally nornicotine and anabasine. Again, this was found to be the case (table 1). The absence of nornicotine in the tomato component of the aerial shoot, incidentally, indicates that little or no transformation of nicotine occurred during its passage through the stem of *N. glauca*. A parallel experiment in which the *N. glauca* component was defoliated gave identical results in that only nicotine appeared in the tomato component of the scion.

As a final check against the possibility that anabasine or some intermediate in alkaloid synthesis may have been translocated downward in these plants from the *N. glauca* scion to the *N. tabacum* root where it could conceivably interfere with the

³ Probably contained a eutectic mixture with anabasine dipicrate (see above).

⁴ The criterion of purity here indicated is constancy of melting point through successive recrystallizations. The melting points of the crystalline products derived from successive recrystallizations in this case when plotted graphically approached $213^\circ\text{C}.$ asymptotically. Insufficient material was available to permit further recrystallization.

normal formation of nicotine, the following experiment was performed. A number of *N. glauca* scions were grown on tomato roots. When these reached a height of eight to ten feet, the tomato roots were separated from the scions and worked up for alkaloids. No anabasine could be detected with picric acid, and only a faint positive test was obtained with silicotungstic acid.

It should be mentioned in connection with the origin of the nornicotine in the grafts between *N. tabacum* and *N. glauca* described herein that numerous attempts to obtain nornicotine from intact plants, both root and shoot, of this strain of *N. tabacum* have failed.

Taken as a whole the evidence described above seems to demonstrate that anabasine production did not interfere in any detectable way with the production by the same plant of nicotine. Rather, the leaves of this strain of *N. glauca* must contain a mechanism similar to that in the leaves of *N. glutinosa* by means of which nicotine is converted to nornicotine following its deposition in the leaf cells. Repetitive experiments have shown no exception to this generalization regardless of the situations imposed. For instance, scions of *N. glauca* grafted to the tops of mature, leafy plants of *N. tabacum* contained the same alkaloids in about the same relative proportions as occurred in similar scions grafted to *N. tabacum* stocks at the ground line.

Alkaloids in the hybrid N. tabacum \times *N. glauca*.—There are many reports in the literature that hybrids of *N. tabacum* and of *N. rustica* (nicotine types) with *N. glauca* (anabasine type) produce anabasine in preference to nicotine in the F_1 generation (see Smith and Smith, 1942, for a review of this literature). With the exception of Smith and Smith (loc. cit.) none of the remaining investigators of this problem have utilized methods of isolation or analysis that could have revealed nornicotine in the presence of anabasine. Smith and Smith detected the presence of this alkaloid in the tissues of one of their hybrids. The significance of their observation, however, was not evident partly because they had also prepared a hybrid from another strain of *N. glauca* in which nicotine occurred in the place of nornicotine (see below).

In the light of the data contained in the present paper on the production of alkaloids in reciprocal grafts of *N. tabacum* with *N. glauca*, it became a matter of some importance to reinvestigate the inheritance of alkaloids in genetical hybrids between these species. Such hybrids were obtained by artificial pollination utilizing *N. tabacum* as the female parent. Attempts to obtain the reciprocal cross failed. Both greenhouse cultures of the intact plants and sterile cultures of the excised roots were established, the latter according to the methods of White (1943).

The leaves of the intact hybrid contained appreciable amounts of nicotine. This alkaloid was isolated both by the use of nitrous acid and by distillation through the Widmer column. The non-

volatile alkaloids were converted to the picrates (m.p. 179–185.5°C.). A portion of this preparation when recrystallized six times yielded impure anabasine dipicrate⁵ (m.p. 205–208°C.). The mother liquors yielded nornicotine dipicrate⁶ (m.p. 182–185.5°C.) and an intermediate fraction (m.p. 191–193.5°C.). Methylation of a second portion of the crude picrate yielded pure nicotine dipicrate after two recrystallizations. The solubility-melting point procedure indicated a ratio of approximately equal parts of nornicotine and anabasine in the mixed picrates after two recrystallizations.

The case for the occurrence of nornicotine is, therefore, clearly established. For further proof of the correctness of the interpretation of these observations the following experiments were performed.

Tomato scions were grafted to hybrid roots. When harvested these scions contained almost equal amounts of nicotine and anabasine. The anabasine was accompanied by small amounts of some impurity (not nornicotine according to solubility-melting-point data) which was largely lost after two recrystallizations (m.p. 209–212°C.). Confirmation of these results was obtained from the working up of seventy-eight cultures of the excised roots of the hybrid. From the combined tissues and spent culture fluids there were again obtained only nicotine and impure anabasine (m.p. 199–200°C.). The data for these experiments are contained in table 1. The theoretical basis for the expected coincidence between results obtained from excised root cultures and grafts involving tomato scions has been outlined elsewhere (Dawson, 1942a).

From the reciprocal grafts (i.e., hybrid scions on tomato stocks) only an extremely small amount of anabasine was obtained. Neither nicotine nor nornicotine could be detected.

Finally, if hybrid scions were grown on *N. tabacum* roots, on the basis of the above information, there might be expected an accumulation of nicotine and nornicotine with only minute amounts of anabasine. Such, indeed, was the case. According to the solubility-melting-point method the distribution of alkaloids in the non-volatile fraction was approximately 98 per cent nornicotine and 2 per cent anabasine dipicrates after two recrystallizations (table 1).

The critical information from the foregoing experiments is summarized in graphic form in figure 2.

DISCUSSION.—The above evidence clearly demonstrates by all-or-none means that nornicotine arises in the leaves of various *Nicotiana* species at the expense of translocated nicotine. In all cases reported herein, this nicotine came from the roots preformed. Hence, the synthesis of nornicotine is secondary in nature: no evidence has been obtained that any or-

⁵ Melting points of successively recrystallized products approached 213°C. asymptotically.

⁶ Probably in part a eutectic mixture with anabasine dipicrate.

gan of the plant is capable of carrying out a primary synthesis of this alkaloid. The nature of the chemical mechanism by means of which nicotine is converted to nornicotine remains to be established. From a consideration of their respective molecular structures, however, the simplest explanation would seem to be that nicotine is acted upon in the cell by a transmethylation agent and a methyl acceptor.

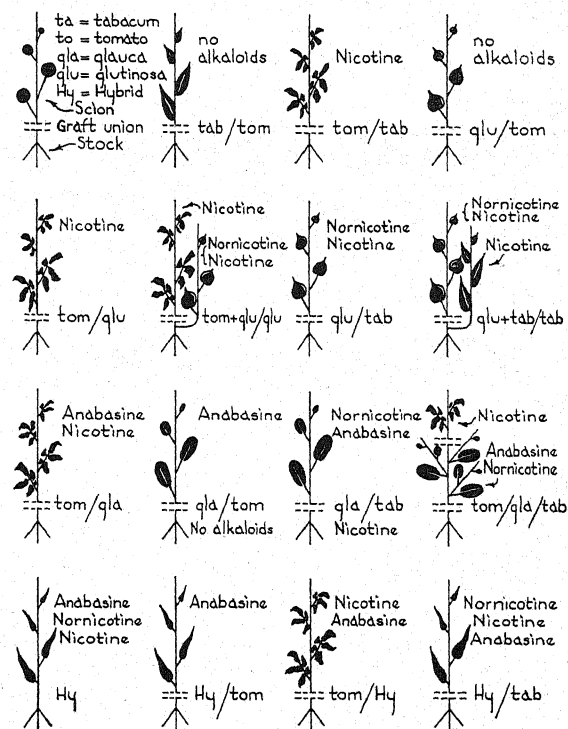
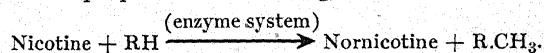


Fig. 2. Accumulation patterns of alkaloids in graft combinations and in hybrids. The alkaloids are listed in the order of their relative abundance in each case.

Transmethylation reactions have been repeatedly suggested to explain the presence of certain compounds in plants (cf. Vickery, 1941). For the purpose of future experimentation in this laboratory, therefore, the following entirely hypothetical mechanism is proposed as a working basis:



Whatever the real nature of the system may be, however, certain of its properties are evident from the results of the present investigation and from the investigations of others. In those strains of *N. glauca* in which the mechanism is present, the overall reaction rate is apparently limited not by the supply of enzyme or of the hypothetical methyl acceptor, but by the very slow rate at which nicotine is formed in the root and delivered to the shoot. Hence, in plants of this strain nicotine never accumulates in detectable amounts. On the other hand those strains of *N. glauca* which do not possess the assumed transmethylation mechanism will

accumulate small amounts of nicotine. This would explain the apparently contradictory results obtained in this laboratory (Dawson, 1944) and by Smith and Smith (1942) regarding the presence or absence of nicotine in intact plants of this species (see above). In the case of *N. glauca* scions on *N. tabacum* roots, however, the rate of delivery of nicotine to the leaves is relatively more rapid and may result in the saturation of the reaction mechanism. Such an explanation may also be extended to the accumulation of nicotine and nornicotine in the hybrid *N. tabacum* \times *N. glauca*, in *N. glutinosa*, in hybrids of *N. glutinosa* with *N. tabacum* (Smith and Smith, 1942), and in grafts of *N. glutinosa* with *N. tabacum*. In other words, the system which is responsible for the transformation of nicotine to nornicotine possesses a limited operating capacity.

A second attribute of the mechanism is that it is inheritable. Such inheritance, indeed, accounts satisfactorily for the predominance of nornicotine in hybrids between nicotine and nornicotine types (Smith and Smith, loc. cit.). With regard to the inheritance of alkaloids in hybrids between *N. glauca* and *N. tabacum*, Smith and Smith have summarized the results of their own and of certain Russian investigations. In those instances in which studies with such hybrids had been carried beyond the first generation, segregation occurred in such a manner that some offspring were found that contained only nicotine, some only "anabasine", and some both alkaloids in various proportions. No obvious Mendelian ratios were encountered. The present work makes it clear that what was inherited and segregated in these experiments was a mechanism contained in the leaves which converted nicotine to nornicotine. It is important to stress the fact that the initial formation of nicotine was not suppressed.

Smith and Smith (1942) also bring up the interesting matter of the relationship of alkaloid inheritance to the identity of the wild progenitors of *N. tabacum*. According to these authors (cf. also Goodspeed, 1934), *N. tabacum* is thought to be derived by amphidiploidy from an F_1 hybrid between *N. sylvestris* and *N. tomentosa* or *N. tomentosiformis*, all of which in the wild state produce predominantly nornicotine. The problem of explaining the development of a nicotine containing species from such a combination when nornicotine production is known to predominate in all artificially prepared hybrids thus far described has been difficult. The results of the present investigation would seem to make an explanation possible. In the first place, since the nature of the inherited character is now more clearly understood, it becomes necessary only to postulate that during the long period of time in which *N. tabacum* has been cultivated growers have selected strains that have lost the transmethylating mechanism. The loss may have occurred by degrees rather than abruptly. The recent discovery by Markwood (1940) that certain Maryland tobaccos which have been selected for their low nicotine content had developed compensating concentrations of nornico-

tine up to 95 per cent of the total alkaloid content may be important in this connection. If this particular case of segregation can be viewed as a possible reversal of the original processes of selection by means of which the high nicotine types were once obtained, then it also becomes possible to suggest that *N. tabacum* may at one time have contained largely nornicotine rather than nicotine as at present. The parallel existence of two strains of *N. glauca* with respect to the presence or absence of the transformation mechanism (see above) is also a case in point. The fact that *N. tabacum* contains higher concentrations of alkaloid than do its wild progenitors may be simply a consequence of its polyploid state. In this connection it is interesting that the absence of one chromosome complement of *N. tabacum* in the hybrid used in the present study resulted in a reduction in the quantity of nicotine produced by the roots, and the absence of one chromosome complement of *N. glauca* resulted in a decrease in the amount of anabasine produced in the shoots (cf. Cain, 1944, p. 21).

Aside from the origin of nornicotine in *Nicotiana*, the present investigation has also shown that there is no valid evidence in favor of the existence of a mechanism by means of which anabasine formation may predominate over the formation of nicotine. In all cases, as has been pointed out above, such assumed interactions can be explained on the basis of the accumulation of nornicotine and the confusion of this alkaloid with anabasine. Hence, hybrids between *N. tabacum* and certain strains of *N. glauca* are of interest largely because they contain within one and the same plant body mechanisms for the synthesis of appreciable amounts of all three of the major *Nicotiana* alkaloids.

Finally, the true nature of alkaloid accumulation in three representative species of *Nicotiana*, *tabacum*, *glauca* and *glutinosa*, with respect to nicotine, anabasine and nornicotine may be outlined. From the point of view of the occurrence of specific alkaloids it is seen that nicotine is produced in the roots only of all three species. Anabasine is produced in both root and shoot of *N. glauca*. Nornicotine is produced in the leaves of *N. glutinosa* and in the leaves of some strains of *N. glauca* and of *N. tabacum*, always, however, at the expense of preformed nicotine. It should be noted, therefore, that either nicotine or nornicotine is to be considered as a normal constituent of *N. glauca* depending upon the particular strain under consideration. Such conclusions strikingly demonstrate the fundamental unity of alkaloid synthesis within the genus *Nicotiana*.

From a broader point of view, the following generalizations may now be made concerning production and accumulation of alkaloids (see also Peacock, Leyerle and Dawson, 1944).

1. The synthetic mechanism for an alkaloid may be localized in a single organ of the plant body, although this is not always so.

2. When synthesis is so localized the newly formed

alkaloid may be transported to other portions of the plant body.

3. Transport of the alkaloid may be followed by accumulation at some distance from the point of initial origin.

4. Transport of an alkaloid may be followed by secondary chemical modifications when the alkaloid molecules are delivered to cells containing the necessary mechanisms.

Although the limited foundation upon which these generalizations are built precludes their immediate extension to the production of alkaloids outside the Solanaceae, it is obvious that their practical consequence in the design of experiments aimed at obtaining such information is great indeed. In this laboratory they are being put to such use in an investigation of alkaloid production and accumulation in the genus *Cinchona*.

SUMMARY

Alkaloid accumulation patterns in graft combinations involving *N. tabacum*, *N. glauca*, *N. glutinosa* and tomato and in the hybrid *N. tabacum* × *N. glauca* have been investigated.

It has been found that nornicotine is produced only in the plant leaf and at the expense of nicotine. This is taken to mean that, insofar as the three major *Nicotiana* alkaloids are concerned, only nicotine and anabasine are produced by total synthesis *in situ*. Nornicotine production is definitely secondary in nature.

It has been shown that, contrary to all reports in

the literature, anabasine production does not predominate over nicotine production in the hybrid *N. tabacum* × *N. glauca*. Rather, nicotine is produced in the roots as might be expected, translocated to the leaves, and there converted to nornicotine. Failure to differentiate between nornicotine and anabasine in mixtures of the two is shown to account for the earlier erroneous reports. The same conclusion is shown to apply to alkaloid accumulation in *N. glauca* scions grown on *N. tabacum* stocks. The demonstration in this case that normal and predictable patterns of alkaloid synthesis and accumulation prevail removes the necessity for explaining the results of grafting experiments by recourse to other than well-recognized biological and biochemical principles.

It has been shown that existing data in the literature concerning the inheritance of *Nicotina* alkaloids in interspecific hybrids other than those discussed above can be readily explained by postulating the inheritance of a mechanism for the conversion of nicotine to nornicotine in the leaves. It is suggested that this mechanism is concerned with a transmethylation reaction.

Finally, a completed picture of alkaloid production, translocation, accumulation and transformation with respect to nicotine, nornicotine and anabasine in *N. tabacum*, *N. glauca* and *N. glutinosa* is given. The inherent unity in these processes is indicated.

DEPARTMENT OF BIOLOGY,
PRINCETON UNIVERSITY,
PRINCETON, NEW JERSEY

LITERATURE CITED

- BOWEN, C. V., AND W. F. BARTHEL. 1944. Classification of tobacco. Ind. Eng. Chem. 36: 475-477.
- , AND —. 1944a. Identification of nornicotine in tobacco. Ind. Eng. Chem., Anal. Ed. 16: 377-378.
- CAIN, S. A. 1944. Foundations of plant geography. 556 pp. Harper and Brothers, New York.
- DAWSON, R. F. 1942. Accumulation of nicotine in reciprocal grafts of tomato and tobacco. Amer. Jour. Bot. 29: 66-71.
- . 1942a. Nicotine synthesis in excised tobacco roots. Amer. Jour. Bot. 29: 813-815.
- . 1944. Accumulation of anabasine in reciprocal grafts of *Nicotiana glauca* and tomato. Amer. Jour. Bot. 31: 351-355.
- . 1944a. Grafting in the Solanaceae. Gardener's Chronicle of America 48: 235 ff.
- GOODSPEED, T. H. 1934. *Nicotiana* phylesis in the light of chromosome number, morphology and behavior. Calif. Univ. Pubs., Bot. 17: 369-398.
- KUZMENKO, A. A., AND V. D. TIKHVINSKAYA. 1940. (In Russian.) Inheritance of nicotine and anabasine content by *Nicotiana tabacum* × *N. glauca* hybrids and interaction of stock and scion when these species are grafted. Bull. Acad. Sci. URSS, Biol. Ser. 4: 564-576.
- MARKWOOD, L. N. 1940. Nornicotine as the predominant alkaloid in certain tobaccos. Science 92: 204-205.
- PEACOCK, S. M., JR., D. B. LEYERLE, AND R. F. DAWSON. 1944. Alkaloid accumulation in reciprocal grafts of *Datura stramonium* with tobacco and tomato. Amer. Jour. Bot. 31: 463-466.
- SHMUCK, A. A. 1940. (In Russian.) Changes in the chemical composition of plants upon grafting. Vsesquiznala akademiia sel'sko-khoziaistvennykh nauk imeni V. I. Lenina, Moscow, Doklady. 11: 9-13.
- , D. KOSTOFF, AND A. BORODZINA. 1939. Alteration in the alkaloid composition due to the influence of stock upon scion in *Nicotiana*. Compt. Rend. (Doklady) Acad. Sci. URSS. 25: 477-480.
- , A. SMIRNOV, AND G. ILYIN. 1941. Formation of nicotine in plants grafted on tobacco. Compt. Rend. (Doklady) Acad. Sci. URSS. 32: 365-368.
- SMITH, H. H., AND C. R. SMITH. 1942. Alkaloids in certain species and interspecific hybrids of *Nicotiana*. Jour. Agric. Res. 65: 347-359.
- VICKERY, H. B. 1941. End products of nitrogen metabolism in plants. Biological Symposia 5: 3-19.
- , AND G. W. PUCHER. 1930. Chemical investigations of tobacco. Connecticut Agric. Exp. Sta. Bull. 311: 234-246.
- WHITE, P. R. 1943. A handbook of plant tissue culture. 277 pp. Jaques Cattell Press, Lancaster, Pa.

MORPHOGENESIS OF FUNGUS COLONIES IN SUBMERGED SHAKEN CULTURES¹

Paul R. Burkholder and Edmund W. Sinnott

WHEN GROWN in liquid media, many kinds of microorganisms, such as bacteria and yeasts, come to be distributed throughout the milieu, and thus the various nutrient materials in solution are made generally accessible to fermentative action. Molds, however, commonly develop floating mats of mycelium in stationary fluid media, the upper surface of the fungus body being exposed to the atmosphere and the lower surface lying in contact with the liquid. The investigations of Kluyver and Perequin (1933) clearly demonstrated the advantages of aeration and mechanical agitation in the cultivation of such molds. They observed in shaken cultures of *Aspergillus flavus* the production of numerous discrete "Mycelkugelchen" distributed throughout the media. By employing shaken cultures they demonstrated the possibility of producing large amounts of cell material with high metabolic activity, in large volumes of nutrient media, and in a relatively short time. Since then numerous investigations have dealt with various aspects of the submerged growth of molds exhibiting characteristic fermentative activity throughout large volumes of media. The results of such studies are evidenced by the present-day methods for commercial production of numerous important metabolic products of bacteria, yeasts and molds. Current techniques employed in maintaining satisfactory distribution of microorganisms in submerged culture include the use of forced aeration, revolving drums, mechanical stirrers, and rotating or oscillating shakers (see Prescott and Dunn, 1940; Herrick, Hellbach and May, 1935). Large

scale operations of this type include the production of alcohols, acetone, various organic acids, penicillin, and numerous other compounds.

Unlike molds cultivated in stationary liquid media which generally produce flat floating masses, fungi grown in agitated culture commonly form numerous, small, discrete colonies which are continuously moved about in the medium. The size, shape, and surface features of these "planktonic" fungus pellets vary with their genetic constitution, and their development is modified to a considerable extent by special environmental conditions. Some observations on the development of submerged colonies of a number of representatives of the several classes of fungi are reported in the present paper, together with a brief discussion of the morphogenetic significance of the results.

MATERIALS AND METHODS.—Pure cultures of fungi obtained from various collections, or isolated from nature, were grown in liquid media in Erlenmeyer flasks on shaking machines. The shakers were of the type which cause the media to perform a continuous rotary or an oscillatory motion. The speed of shaking was adjusted by means of reduction gears and pulleys to about 100 complete cycles per minute. The cultures were grown in a darkened room, and the temperature was kept at approximately 25°C during the growing periods, which varied in length up to two weeks. Fifty milliliters of medium contained in each cotton-stoppered 125 ml. flask were sterilized by autoclaving at 15 pounds for 15 minutes. Inoculation was accomplished with a sterile wire loop by transferring small portions of my-

¹ Received for publication April 9, 1945.

TABLE 1. Composition of nutrient solutions used for fungi.

Constituents	Synthetic medium	Yeast extract medium	Czapek corn-steep medium
NaNO ₃	2.0 gm.
KH ₂ PO ₄	1.5 gm.	1.0 gm.
KCl	0.5 gm.
MgSO ₄ ·7H ₂ O	0.5 gm.	0.5 gm.
FeSO ₄	0.01 gm.
Glucose	60.0 gm.	20.0 gm.	40.0 gm.
Corn-steep liquor	20.0 ml.
Yeast extract	5.0 gm.
Asparagine	4.0 gm.
Trace elements	see footnote ^a
Thiamine . HCl	200.0 microgm.
Pyridoxine . HCl	200.0 microgm.
Niacin	200.0 microgm.
Ca pantothenate	200.0 microgm.
Inositol	10,000.0 microgm.
Biotin	2.0 microgm.
Distilled water	To make 1 liter	To make 1 liter	To make 1 liter

^a Trace elements were added to this medium in p.p.m. as follows: B, 0.1; Mn, 0.01; Zn, 0.07; Cu, 0.01; Mo, 0.01; and Fe, 0.05.

celium or groups of spores from agar slant cultures. Three kinds of media were employed: (a) synthetic medium, (b) yeast extract medium, and (c) Czapek's nutrient supplemented with corn-steep liquor. The composition of each of the media used is shown in table 1.

When the cultures had attained satisfactory growth, typical colonies of each species were removed for observation under a binocular microscope. For purposes of making photographic records, colonies were floated in water, illuminated unilaterally with two spotlights, and photographed with a 72 mm. microtessar lens on process film. A few photographs were made at higher magnification with microscope objectives and a series of oculars. Some young stages were mounted in lactophenol, and a few colonies were imbedded in paraffin, sectioned with a microtome, and stained with carbol-fuchsin.

RESULTS.—Variation among species.—The gross morphology of approximately 150 species of fungi was observed in shaken submerged cultures. Rapid growth of most of these fungi was found to occur in yeast extract medium; hence this nutrient solution was employed in the general study of the development of colonies. Some of the differences in external appearance of selected species is illustrated in figure 1. Considerable variation was observed in the character of the colonies. Some developed a smooth surface, others were markedly hirsute; some were compactly organized, others loosely held together or entirely lacking in colonial organization. The texture of the colonies was often sponge-like, yielding to pressure with exudation of capillary liquid held in the intermycelial spaces. Upon removing mechanical pressure, the original spheroidal form was often restored and liquid was again taken into the capillaries between the elastic strands of intertwined mycelial threads. The form of the colonies generally was globose, although in some species there was considerable departure from the usual spheroidal shape. A strain of *Neurospora crassa* known as "Star" developed irregularly-branched colonies, and showed no tendency to form spheres (fig. 1, no. 14). Many species of *Fusarium* tended to be very poorly organized, the mycelium breaking into small fragments. Several species of *Fusarium*, however, were observed to form globose colonies, e.g., *Fusarium niveum* (fig. 1, no. 11). Many species of fungi developed branching strands of mycelium which radiated loosely from a common center.

Except in the genus *Fusarium*, only a few kinds of fungi appeared unable to organize definite colonies, e.g., *Phomopsis vexans* (fig. 1, no. 19), *Cercospora apii*, and *Verticillium albo-atrum*. In some instances, colonies of irregular form showed evidence of possessing more than one center of growth, as for example certain cultures of *Basidiobolus ranarum* (fig. 1, no. 1) and *Penicillium roqueforti*. Globose colonies with almost smooth surfaces were formed by *Basidiobolus ranarum*, *Fusarium solani*, and *Peni-*

cillium brevicaulis. Somewhat less smooth in appearance were the colonies of *Agaricus campestris* (fig. 1, no. 5), *Aspergillus flaviceps*, *A. giganteus*, *A. ochraceus* (fig. 1, no. 3), *A. versicolor*, *Chaetomium caprinum*, *C. olivaceum*, *Cunninghamella Bainieri*, *Gliocladium roseum*, *Morchella crassipes*, *Myceliophthora lutea*, *Poria undora*, *Trametes heteromorpha*, etc. Colonies which to the naked eye appeared to be covered with short hair-like projections include such species as *Absidia glauca*, *A. spinosa*, *Aspergillus ochraceus*, *A. terreus*, *A. Wentii*, *Dactylium dendroides*, *Guignardia bidwelli*, *Lenzites trabea*, *Macrosporium sarcinaeforme*, *Penicillium chrysogenum*, *P. camemberti*, *Phycomyces Blakesleeana* (fig. 1, no. 4), *Pleurotus corticatus*, *Poria subacida*, *Rhizopus oryzae*, *Trametes serialis*, etc. Species which tended to produce long hair-like radiating hyphae over the surface of the colonies are the following: *Cephalosporium ulmi*, *Fomes pinicola*, *Hypophoma fasciculare*, *Gibberella saubinetii* (fig. 1, no. 15), *Polyporus sulphureus*, *Sordaria fimicola*, etc. In some species the colonies were composed of very loosely organized radiating hyphae, e.g., *Conidiobolus Brefeldianus*, (fig. 1, no. 17) and an unidentified marine mold. The general appearance of three species of marine molds is shown in figure 2, numbers 17, 18, and 19. The tendency to form one large irregular mass was observed in *Mucor griseocyanus*, *Mucor racemosus*, *Rhizopus suinus*, *Zygorhynchus vuilleminii*, and *Zygorhynchus Dangeardii*. The very rapid growth made by these fungi may explain the coalescence of the entire growth into one fungus body. Small inocula of these species in larger volumes of nutrient solution might permit the development of discrete colonies. Although there are numerous exceptions, it is apparent that essentially spherical colonies with various types of surface features represent a common pattern of development in the submerged growth of numerous species belonging to widely different taxonomic groups.

Internal structure of the colonies.—The tissue structure of the colonies was generally prosenchymal with somewhat varied texture, the hyphae being loosely arranged or closely packed together. Most species formed relatively loose prosenchyma consisting of branched hyphae radiating outward from the original center of growth, and with large, continuous, intercellular spaces filled with culture fluid. Generally the outermost region was composed of hyphae intertwined so as to form a kind of toughened cortex. Only rarely was this cortex differentiated as a pseudoparenchyma.

A transection and an external view of a species of *Mucor* shown in figure 2, numbers 11 and 12, illustrate the usual appearance. In this median section, cut at 120 microns with a microtome, the central region of the colony is seen to consist of closely packed hyphae which exhibit great affinity for carbol-fuchsin. The flat disc of aerial mycelium which was the original inoculum is evident in the central deeply stained core which had early grown into the form of

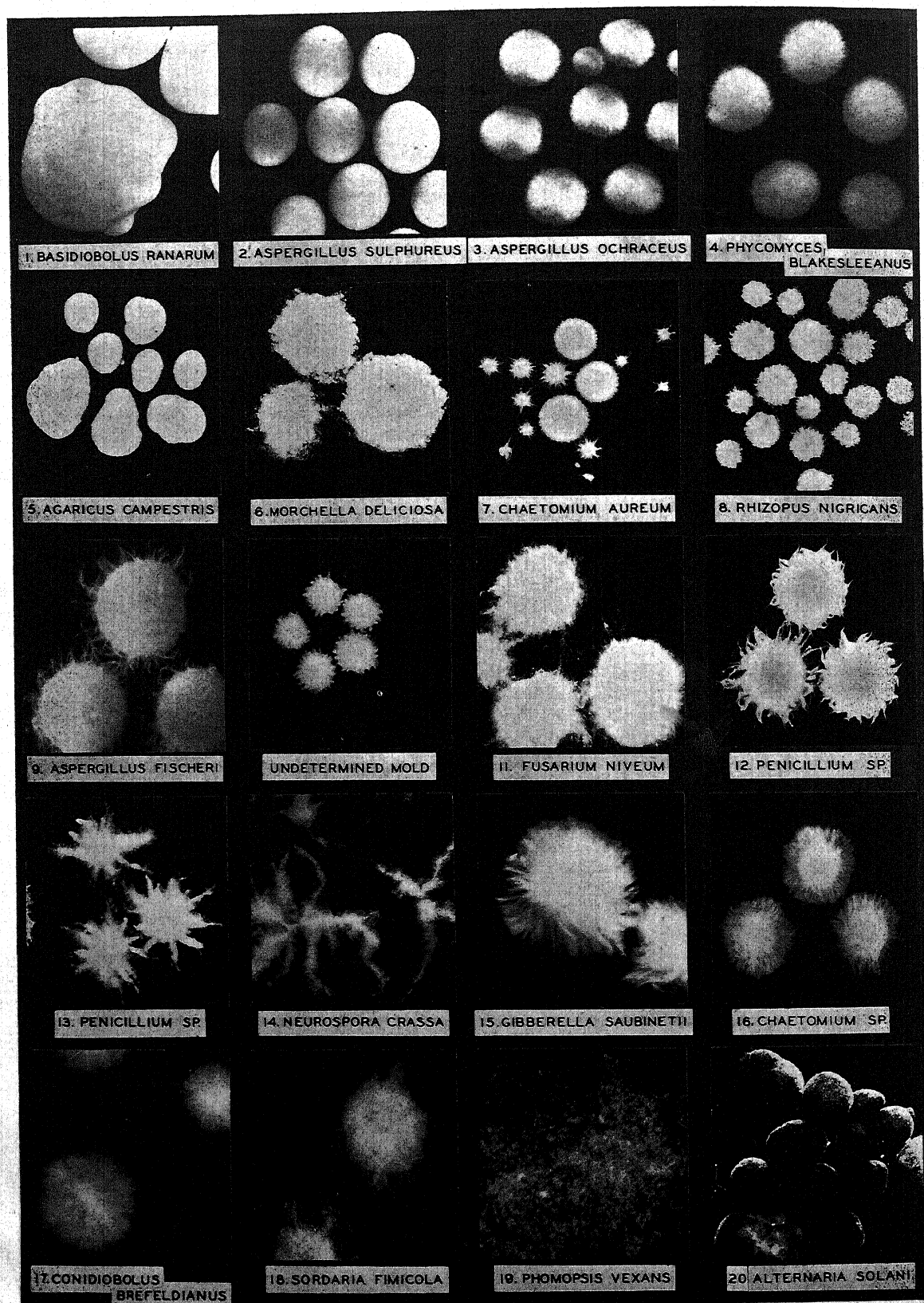


Fig. 1. Colonies of fungi grown in yeast extract dextrose solution on a shaking machine. Magnification $\times 2.5$. Note the diversity of pattern and surface texture.

a sphere. The greater part of the volume of the colony consists of loosely woven hyphae, surrounded on the outside by a cortex. With the exception of the dense central core, which is usually absent from colonies of many species of fungi, this cross sectional view is fairly typical of the colonial structure of molds grown in submerged culture. Further observations were made on the internal colonial structure of a number of species, using freehand sections and paraffin sections, and also by dissecting the colonies with needles under a low power binocular microscope.

In general the fungi which were grown in shaken culture for periods up to 10 or 14 days are characterized by vegetative growth, few species showing any reproductive structures. Reproduction by spores was observed, however, in *Alternaria solani* (fig. 1, no. 20), *Aspergillus niger*, and *Penicillium roqueforti*. The colonies of *Alternaria* and *Aspergillus* turned black, and the mold from roquefort cheese developed colonies colored deep green by the spores. Typical spore clusters of *A. niger* were distributed at random throughout the interior of the colony, and the spores apparently were discharged into the intercellular spaces.

The texture of many species was very tough. Others like *Phycomyces Blakesleeanus* appeared to be held together weakly. The loosely woven strands of this species tended to stretch and part irregularly when subjected to violent shaking at increased speeds. Under ordinary conditions of shaking these parted fragments continued their growth and developed into globose colonies. Although nearly all species included in this study were white or light gray in appearance, in a few the formation of various pigments, yellow in certain strains of *Phycomyces* and in other genera, orange and purple in species of *Fusarium* and in *Chaetomium*, was very striking. Strong odors were occasionally observed in certain species.

Developmental history of shaken cultures.—For the purpose of determining progressive stages in the development of form, the well known *Penicillium notatum* was selected for special observations at different periods of time. Groups of spores were inoculated into three kinds of media, and after various intervals samples were taken for study. Some selected photomicrographs shown at the top of figure 2, numbers 1–7, indicate the appearance of the sporelings, and young and old colonies which developed in Czapek corn-steep medium. The photograph numbered 1 was taken about 8 hours after inoculation, 2 after about 18 hours, 3 after 24 hours, 4 and 5 on the second day, 6 on the 6th day, and 7 after 10 days. Growth and development of colonies takes place rapidly from the germination of a cluster of spores. In *P. notatum*, the germ tubes radiate outward from the group of spores and soon are held together firmly by the interlacing branched mycelium. *Penicillium* and other molds were observed to grow into spheres as large as one inch or more in diameter under con-

ditions where ample volumes of medium were employed for cultivation of relatively few colonies.

Spherical colonies also develop regularly from single spores, but the time required for their appearance is somewhat longer than in the case of colonies derived from groups of spores. The germinating single spore develops a filamentous germ tube. The strict axial form of the sporeling due to its strongly polarized growth is soon changed to a pattern of branching hyphae which results in the production of a plant body spheroidal in form. This globose mass of radiating hyphae then follows the general sequence of development outlined above for colonies which arise from groups of spores.

The influence of delayed shaking on the form of the colonies is illustrated by two species of *Penicillium*. Czapek corn-steep medium was inoculated with spores of fungi and some flasks were placed on the shaker immediately, while others were kept stationary for a period of about 15 hours until after spore germination had occurred and growth of the mycelium was well advanced. The latter were then placed on the shaking machine and all were subjected to continuous shaking for 6 days. The influence of delay in the beginning of shaking is shown by the irregularly formed colonies of *P. notatum* in figure 2, number 9. Colonies formed in the same medium shaken from the time of inoculation are spherical in form (fig. 2, no. 6). Another species, *P. commune*, formed hirsute spheres in continuously shaken culture (fig. 2, no. 15) and irregular colonies in cultures subjected to delayed shaking (fig. 2, no. 16). It appears that early growth of a sporeling or groups of sporelings, when in still culture, may produce so irregular a pattern that during subsequent shaking a regular globose form is not developed. Perhaps organization of spheres would occur eventually if growth of these irregular colonies were allowed to progress over a much longer time. These observations indicate that form of colonies is determined, at least in some instances, in comparatively early stages of development.

Effect of environmental conditions.—The production of varied features of texture and surface in globose colonies of fungi in shaken culture was investigated briefly in relation to the composition of the medium, as represented in the three types of culture solutions listed in table 1. A fact thus far observed only in a limited number of molds is illustrated by two examples in the genus *Penicillium*. *P. notatum* grown in shaken culture for ten days differs strikingly in two different kinds of media, as shown in figure 2, numbers 7 and 8, for rough colonies were produced in Czapek corn-steep medium and comparatively smooth ones in synthetic medium. *Penicillium digitatum* produced loosely-organized, mycelioid colonies in yeast extract medium (fig. 2, no. 13), and relatively compact spheres in Czapek corn-steep medium (fig. 2, no. 14). Whether these observed differences in development are to be attributed to differences in physical conditions or chemi-

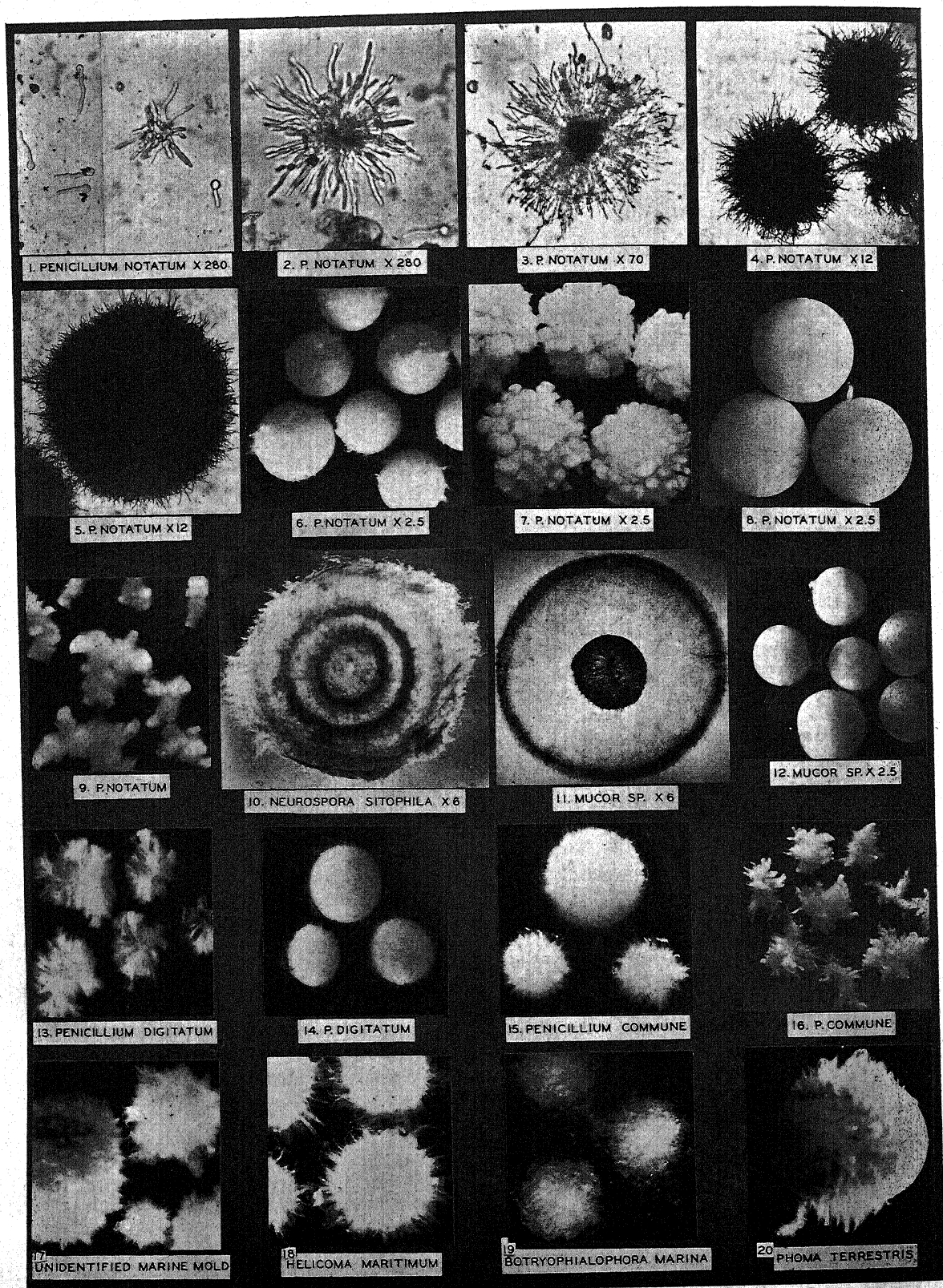


Fig. 2. Colonies of fungi grown in shaken cultures. Magnification $\times 2.5$ unless stated otherwise. Nos. 1 to 7 show the developmental history of *Penicillium notatum* from spore germination to the formation of mature rough colonies

cal constituents of the different media is not known. It seems clear, however, that specific texture and surface features of colonies produced in submerged culture depends, in some instances, upon properties of the medium.

Several species of fungi were grown in stationary culture as deep colonies enclosed in nutrient agar in large Petri dishes. The form in these cases was globose with typical radiating hyphae and a general appearance similar to that of shaken colonies. This fact, which is familiar to experimental mycologists, indicates a relation between uniform chemical environment and the development of globose colonies of molds.

The influence of temperature changes upon internal differentiation was observed in a few species. Growth was allowed to take place at 25°C on the shaking machine for 48-hour periods alternating with 48-hour periods when the cultures were kept stationary at 0°C. This treatment resulted in the formation of alternating spherical shells of loosely and of compactly developed mycelia as the colony increased in size under favorable temperature conditions and ceased growth during periods of subjection to low temperature. Enlargement of the colonies appears to take place by outward growth of the hyphae in the peripheral regions. A freehand median section, unstained, taken through a living colony of *Neurospora sitophila* illustrates the general appearance of the alternating zones (fig. 2, no 10). Shaken cultures of *Penicillium chrysogenum* which had developed typical globose colonies were allowed to grow for one day under stationary conditions. The free-floating colonies then showed very rapid radial growth at the surface so that the appearance became similar to that shown in figure 1, number 16 for a species of *Chaetomium*. Shaking was then resumed for two more days, and a more firm texture was again produced in the cortical region of the colonies. Freehand sections through these colonies revealed alternating zones of compactly and loosely developed tissues. It seems probable that fluctuation of any environmental factor which affects the rate of growth might be expected to produce some modification in the internal pattern of development.

DISCUSSION.—The fact that molds when grown in shaken culture form essentially globose colonies and that these differ in character in different types and under various environmental conditions presents a number of interesting morphogenetic problems.

The spherical form is found occasionally in al-

gae, as in *Gloeotrichia*, *Rivularia*, *Volvox* and some species of *Cladophora*. In fungi it occurs in many sporangia (Mucorales and others) and sometimes in larger aerial fructifications, as in the puffballs. In such cases it seems to result from turgor in a body bounded by a firm membrane or rind. The spheres described in the present paper, however, seem to be in quite a different category. Although the colony as a whole is globose, the individual hyphae, at least in most cases, are freely exposed to the medium, and there is no special differentiation of the outermost layer into a definite tissue. These facts indicate that the spherical form here is not a least-surface phenomenon.

Two other possible explanations for it suggest themselves. The spheres may be the result of constant mechanical contacts between the colonies and their neighbors or the sides of the flask. This would be plausible if all species showed the firm, smooth surface of *P. notatum* as shown in figure 2, number 8. In many, however, the colony consists of delicate hyphae, even at the surface, and could hardly have reached this form as the result of long-continued battering. That abrasion is sometimes effective in producing a smooth surface is shown in figure 2, number 20, a large colony of *Phoma terrestris*, in which tufts of hyphae on part of the colony were apparently unable to develop because of friction against the walls of the culture vessel. It is noteworthy that the spherical form begins very early, when the colony consists simply of a group of radiating hyphae (fig. 2). Perhaps thigmotropic stimulation may be a more important factor than simple mechanical contact, and by producing equal growth over the whole mycelial surface may lead to the formation of spherical masses.

Another hypothesis seems more in accord with the facts, namely that the spherical form is simply the result of equal growth in all directions, due to the elimination of orienting factors in the environment, as a result of shaking, which under these conditions are no longer able to act in a unilateral fashion on the colony. In the absence of such factors, any growing organic system will tend to become spherical. That so few plant forms are spheres is testimony to the importance of such factors in development. The mycelium in still culture, growing as a flat plate near the surface of the medium, is exposed to the unilateral action of a number of these factors. These would be eliminated or neutralized by shaking.

One such factor is gravity. The rolling and tumbling mycelial colonies are being continually

in Czapek corn-steep medium; no. 8 illustrates the appearance of smooth colonies of *P. notatum* grown in synthetic nutrient; no. 9 illustrates the effect of delayed shaking on *P. notatum*; no. 10 is a median section through a globose colony of *N. sitophila* which had been subjected to alternating conditions; no. 11 is a section through a colony of *Mucor* (no. 12 is the exterior) showing some structural differentiation of the vegetative mass; no. 13, *P. digitatum* grown in yeast extract medium; and no. 14, the same species in Czapek corn-steep medium; no. 15, *P. commune* grown in continuously shaken medium and no. 16 in the same medium shaken only after the first 15 hours; nos. 17, 18, 19, some marine molds grown in yeast extract solution; no. 20, *Phoma terrestris* colony showing smooth areas resulting from friction against the walls of the culture vessel.

changed in their orientation to gravity, just as though they were on a clinostat, revolving not on a single axis but on a universal joint. Filamentous fungi in many cases show geotropic reactions, and it is possible that the flat mycelial mass in still culture may owe its form, in part at least, to gravity. The development of globose colonies in deep agar in still cultures, however, suggests that gravity is not a determining factor of primary importance.

Other orienting factors which shaking would largely eliminate are chemical gradients of various sorts. A still culture has much more ready access to oxygen on its upper than on its lower surface. Differences in concentration of nutrilites, resulting from absorption at the hyphal surfaces, would largely disappear with shaking, a fact which seems to be responsible for much of the increased growth in stirred or shaken cultures. Gradients in metabolic products given off by the hyphae would also be minimized. The importance of chemical gradients in the orientation of growth in lower plants has frequently been shown, as in the effect of pH gradients on the direction of rhizoid formation in the germinating *Fucus* egg (Whitaker and Lawrance, 1937).

A third orienting factor which shaking might remove is the unequal distribution of materials within the cells themselves. How much these materials might be affected by shaking would depend on the viscosity of the cytoplasm and other factors, but the ease with which many visible substances in cells may be moved about by centrifugation suggests that the vigorous agitation to which these hyphal cells have been subjected may well result in a more uniform distribution of their contents, including those substances which are active in determining the direction of growth. The more subtle details of cytoplasmic structure itself, which apparently control the direction of movement of hormones and similar materials, might also be altered by shaking.

To determine which of these factors are the ones eliminated by shaking, and whether there are others, will require further experiment. It should be possible, for example, to grow a mycelial colony on agar rotated on a clinostat to determine the effect of gravity. Colonies implanted on the surface of firm agar and then continuously shaken might affect the distribution of materials within the cells and thus throw light on the importance of such distribution in producing oriented growth.

The factors here discussed are general and non-specific. If they were the only ones concerned in the form of the mycelial colony, these colonies would all look alike. The many differences in color, surface and texture, however, are very evident from a comparison of the illustrations in figures 1 and 2. Apparently there are other factors operating here than the simple non-specific ones previously mentioned. The cells themselves are different in the different species. These differences presumably originate in the living material and are genetic in character. They are expressed directly in such qualities

as color, surface and texture, which do not depend on orienting factors in the external environment.

Specific differences among these fungi in the form of the colony when grown in still culture are not conspicuous, since the colony is a loosely organized thalloid mass. Other organisms, however, such as many algae, have attained definite forms of body or colony and can be cultured in the manner here described. It would be instructive to grow some of these in shaken culture to find what alteration in form might result.

The technique of shaking should thus prove a useful morphogenetic tool for the study of any organic structure which can be grown in liquid media. By its means the organism or colony is exposed on all sides equally to various factors in the external environment and its growth can be compared with normal stationary individuals upon which these factors act only in a unilateral fashion. Many characters seem to be due to specific reactions of the organism to such orienting factors and these characters can be identified as the ones which are radically altered when growth occurs under equal exposure to environmental factors. Differences which persist in shaken cultures (as surface characters in the types shown in figure 1) are evidently determined without regard to orienting or unilateral factors in the external environment. Some of these may be due to the general character of the external environment (apart from its orienting effect) as in the difference between *Penicillium notatum* grown in Czapek corn-steep medium and in synthetic medium (fig. 2, nos. 7 and 8) and between *Penicillium digitatum* grown in yeast extract medium and in Czapek corn-steep medium (fig. 2, nos. 13 and 14). Still other characters may be due strictly to factors in the internal environment alone, presumably controlled more directly by the genetic constitution of the nuclei.

Every organic trait is the result of a reaction between the genetic makeup of an individual and its environment. The advantage of the use of shaken cultures with liquid media of various types is that by this means it is possible to distinguish three types of such reactions: (1) reactions to unilateral, orienting factors in the external environment (such as gravity, unilateral light and chemical gradients); (2) reactions to general or unoriented factors in the external environment to which the organism is exposed equally on all sides (such as particular temperatures, chemical substances and quality of light); and (3) reactions to factors in the internal environment alone (such as hormones, enzymes and other substances controlled directly by gene action). The specificity of the reaction in each type would depend on the genetic constitution of the organism. Ability to disentangle these three types of interaction between the developing organism and its environment would be of great usefulness in gaining a clearer understanding of morphogenetic mechanisms.

SUMMARY

Observations were made on the development of submerged colonies in shaken cultures of about 150 species of fungi grown in three kinds of nutrient solution. In still cultures, most fungi form mats of mycelium floating at the surface of the nutrient solution; but in agitated cultures, they develop discrete globose colonies which commonly possess characteristic metabolism, color, texture, and surface features. The tissues of these colonies are usually prosenchyma, elastic, and sponge-like, with fluid filling the interhyphal spaces. Typical colonies may develop from germinating single spores or groups of spores, from fragments of mycelium, or from whole perithecia, etc. Polarized growth of sporelings gives way in early stages of development to branched hyphae which continue to grow outward and intertwine in such a way as to form a globose body. The developmental history of *Penicillium notatum* was studied at various stages of growth from spore germination to the formation of large colonies. The rate of growth, texture, and characteristic surface features of fungus colonies varied considerably with differences in composition

of the medium. Alternating temperature, and intermittent shaking resulted in the development of alternating zones of different texture. Delayed shaking resulted in the formation of colonies with irregular shape.

It is suggested that the development of spherical colonies results from their equal exposure on all sides to various factors, such as gravity and chemical gradients, which in still culture would be unilateral in incidence and effect. The genetic constitution of an organism, as illustrated by these plants, evidently reacts specifically to three types of environmental influences: (1) external factors to which it is exposed on one side more strongly than on the other and which thus tend to produce oriented or unsymmetrical growth; (2) external factors to which it is exposed equally on all sides but which by differences in character or quality produce diverse effects; and (3) factors in the internal environment more immediately under genic control and relatively independent of external influences.

DEPARTMENT OF BOTANY,
YALE UNIVERSITY,
NEW HAVEN, CONNECTICUT

LITERATURE CITED

- HERRICK, H. T., R. HELLBACH, AND O. E. MAY. 1935. Apparatus for the application of submerged mold fermentations under pressure. *Ind. Eng. Chem.* 27: 681-683.
- KLUYVER, A. J. AND L. H. C. PEREQUIN. 1933. Zur Methodik der Schimmelstoffwechseluntersuchung. *Biochem. Zeitschr.* 266: 68-81.
- PRESCOTT, S. C. AND C. G. DUNN. 1940. *Industrial microbiology*. 541 pp. McGraw-Hill, New York.
- WHITAKER, D. M. AND E. W. LAWRENCE. 1937. The effect of hydrogen ion concentration upon the induction of polarity in *Fucus* eggs. *Jour. Gen. Physiol.* 21: 57-70.

A MORPHOLOGICAL, DEVELOPMENTAL, AND CYTOLOGICAL STUDY OF FOUR SAPROPHYTIC CHYTRIDS. I. CATENOMYCES PERSICINUS HANSON

Anne Marie Hanson¹

THE TRUE chytrids with posteriorly uniflagellate zoospores were formerly believed to be relatively few in number, but recent studies have brought to light so many new genera and species that our concepts of the group have undergone marked changes. This is particularly true in relation to the saprophytic species which occur on decaying vegetable and animal matter in the soil. Since 1935 no less than twenty-four new genera and eighty-seven species have been discovered. The multiplicity of forms so far found in the soil indicates that an almost unlimited microflora still awaits discovery.

The present and following series of contributions add two new genera and four new species to the increasing list of chytrids. Of these four, *Catenomyces persicinus*, *Rhizophydium coronum*, and *Catenochytridium laterale* were briefly diagnosed in a previous note by the author (1944). The fourth spe-

cies apparently represents a new group for which another genus must be created. The first paper of this series deals primarily with the morphology, organization, and developmental cycle of *Catenomyces persicinus*.

This species was first discovered in October, 1943, in bits of cellophane which had been floated on a water sample collected in Quaker Brook near Sharon, Connecticut. Attempts to culture and isolate this fungus from other chytridiaceous species growing with it were begun at once. Fragments of cellophane containing portions of the rhizomycelium were rinsed in distilled water and placed in charcoal water, distilled water, and also in water from Quaker Brook, which had been sterilized by filtration. None of these liquids by themselves proved satisfactory. Within twenty-four hours growth was inhibited in both charcoal and distilled water. Deterioration of the protoplasm of the rhizomycelium took place within two to three days. In filtered brook water deleterious effects became

¹ Received for publication April 10, 1945.

The writer wishes to express her gratitude to Professor John S. Karling for his encouragement and stimulating criticisms during the course of this work.

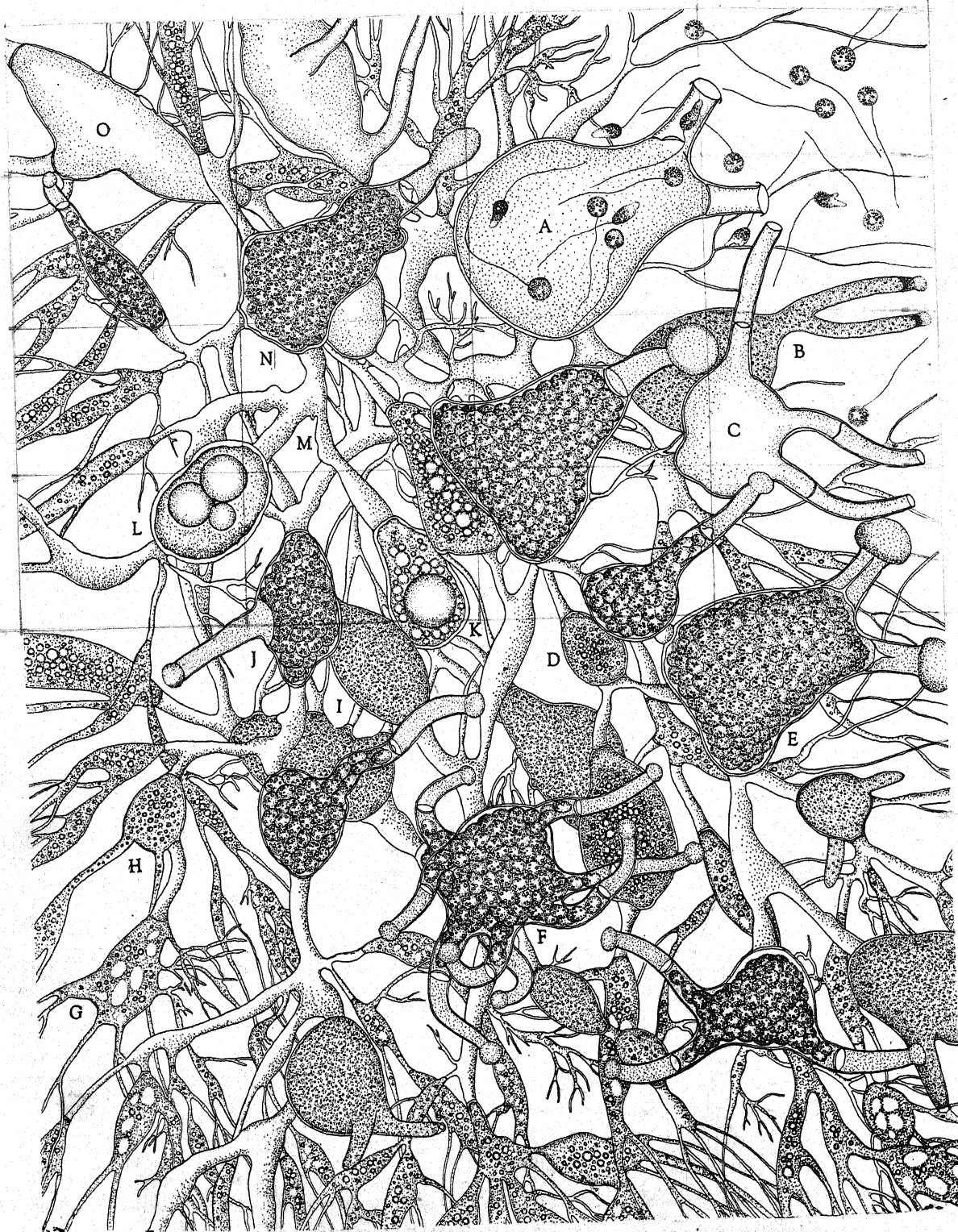


Plate 1. Fig. 1. Portion of an extensive thallus of *Catenomyces persicinus*, with sporangia in various stages of development, and also two thick-walled cells which may be resting bodies. $\times 525$. All drawings were done with the aid of a Zeiss drawing prism and $\times 10$ compensating oculars and enlarged by means of a pantograph.

equally pronounced in four to six days. However, when fragments of cellophane containing the rhizomycelium with mature sporangia were placed in brook water, reinfection of freshly added cellophane sometimes occurred. The newly established rhizomycelia, however, did not reach maturity. Accordingly, the complete cycle of the chytrid could not be determined by this culture method.

Soil extract was next tried as the liquid medium. It was prepared by adding distilled water to soil collected in the vicinity of Quaker Brook. After one or two days the liquid was decanted, filtered and autoclaved for twenty minutes. Growth of *Catenomyces* in bits of cellophane suspended in this medium was profuse and vigorous, but the best growth occurred when an equal quantity of charcoal water was added to the extract. Once a satisfactory liquid was found, this chytrid could be readily isolated. By transferring a single mature sporangium to fresh soil extract containing numerous bits of cellophane, sparse unichytrid cultures were established. In such, fragments bearing only one thallus were readily found. Such single thalli have their origin from one zoospore, and cultures derived from them were accordingly monozoosporic. Sterile dried onion skin, bleached corn and grass leaves were also used as substrate, but inasmuch as growth was sparse, they were not suitable for the maintenance of stock cultures.

STRUCTURE AND GERMINATION OF THE ZOOSPORE.—The living zoospore (fig. 2-5) is spherical, 3.7-4.5 μ in diameter, and contains from five to eighteen golden refractive globules which may be nearly uniform or variable in size. In some zoospores one of the globules may be much larger than the others (fig. 4), suggesting perhaps that fusion of the smaller globules has taken place. This process invariably occurs at the time of germination. Besides these globules, very little is to be seen in the active living zoospore. In fixed and stained preparations, however, the nucleus occupies a somewhat posterior position, and around its upper periphery a zone or cap of dense staining material is often present, as is shown in figures 12 and 13. This zone is similar in size, shape, position, and staining reaction to the nuclear cap which has been described in certain other chytrids (Karling, 1937; Hillegas, 1940; Ajello, 1942) and species of the Blastocladales (Couch and Whiffen, 1942).

During the active swimming period, which may last from twenty minutes to two hours, the zoospores are intermittently amoeboid (fig. 7-9). In the amoeboid phases almost any shape may be assumed. The pseudopodia which are thrust out may be very delicate and attenuated, and strikingly similar to the pseudopodia figured by Karling (1934) for *Catenaria anguillulae*. The refractive globules never migrate out into the pseudopodia, but always remain closely aggregated at the posterior end of the amoeboid spore (fig. 7-9). The flagellum which is about 30 μ long is dragged along posteriorly and

may be easily measured at this stage. When spores are killed with osmic acid fumes, the flagellum stains readily with Plimmer and Paines' modification of Cesares-Gil's method. In accord with the studies of Couch (1941), the flagellum is differentiated into a long thick basal part and a thin short lash or tailpiece (fig. 12).

Toward the end of the swimming phase a distinct hyaline vesicle is frequently formed terminally on the flagellum. The spores are active when the vesicle first appears and the latter seems to interfere considerably with flagellar motion. The spore swims along at a greatly reduced speed, stopping for considerable intervals and jerking the flagellum about with apparent effort, as if the vesicle were a weight on the flagellum. The terminal vesicle (fig. 10) gradually enlarges, and as it does so the length of the flagellum decreases. Eventually the flagellum disappears as such and is completely replaced by an enlarged vesicle which is apparently made up of flagellar material (fig. 11). Such spores usually come to rest, but even in this stage they may still exhibit feeble motility. Although they move about, they do not migrate very far, and soon all movement ceases. The eventual fate of the vesicle was not definitely determined, but since its formation is similar to that described by Karling (1944b) for *Rhizidium verrucosum*, it is possible that the vesicle is absorbed as the spore germinates.

In addition to the normal-sized zoospores, dwarf and giant ones are frequently formed with two to six flagellae (fig. 5, 6). These are probably abnormal spores resulting from unequal or incomplete cleavage. In the spores the position of the nucleus is indicated by a clear area. In normal-sized spores (fig. 2-4, 7-11), one clear area is visible surrounded by refractive globules. In larger multiflagellate spores (fig. 6) more than one clear area is evident. This suggests the presence of more than one nucleus in such spores.

The zoospores usually swell up and degenerate in water and rarely germinate under such conditions. When they do, a thin germ tube is formed which grows only a short distance. On bits of grass or cellophane, however, the zoospores germinate readily, and in so doing the germ tube may either penetrate the substratum directly or grow along its surface for a short distance. While this is going on, the refringent globules in the spore body coalesce to form larger ones, but within a short time this refractive material begins to disperse, so that by the time the spore body is almost empty, very little globular substance is visible. The intramatrical germ tube is usually quite thick and blunt (fig. 17) and, as is shown in figures 18 to 22, it may often enlarge directly into the primary sporangium. In other instances (fig. 23-25), the primary sporangium arises from branches of the initial germ tube. Commonly, the germ tube upon entrance into the cellophane grows along horizontally just beneath the surface, and branches arise from it both above

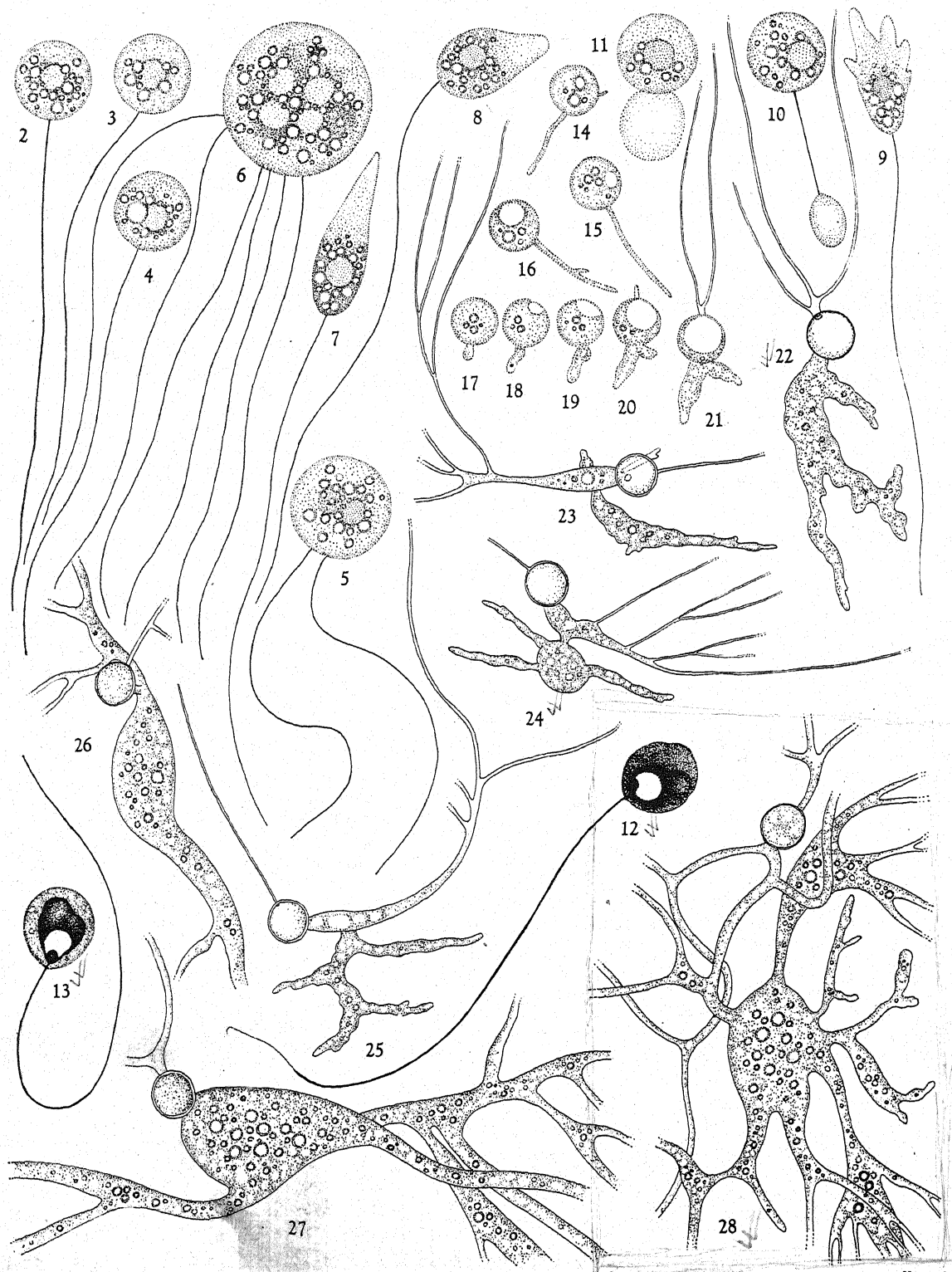


Plate 2. Fig. 2-4. Actively swimming zoospores. $\times 5040$.—Fig. 5, 6. Giant zoospores with two and six flagellae. $\times 5040$.—Fig. 7-9. Amoeboid zoospores. $\times 5040$.—Fig. 10, 11. Zoospores with flagellar loops. $\times 5040$.—Fig. 12. Stained zoospore with lash or tailpiece terminal on the flagellum. $\times 5040$.—Fig. 13. Stained zoospore showing nuclear cap. $\times 5040$.—Fig. 14-16. Germination of zoospores on cellophane. $\times 2520$.—Fig. 17-22. Germination of one zoospore which has penetrated the cellophane. $\times 2520$.—Fig. 23-25. Germination of spores, showing branching of germ tube and the origin of extramatrical hyphae. $\times 2520$.—Fig. 26-28. Relationship of the old zoospore case to the incipient primary sporangium and its branches. $\times 2520$.

and below. The branches that arise above emerge through the cellophane into the surrounding liquid. They are delicate, branched or unbranched, and sterile. The branches that originate from the lower surface of the germ tube grow deeply into the cellophane, and establish the intramatrical rhizomycelium (fig. 23, 24),

Sterile extramatrical hyphae also may arise from the zoospore itself (fig. 20-25). In addition, during the development of the intramatrical rhizomycelium, fine branches are now and then sent up into the liquid medium, and in this manner a dense matting of sterile hyphae is often formed which covers the upper surface of the cellophane. Figure 25 shows a young thallus developed from a germ tube which failed to enter the substrate but nevertheless sterile hyphae arose at the distal end of the germ tube and a branch was given off below which penetrated the cellophane. The zoospore case (fig. 22-28) remains as a thick-walled cyst throughout development of the thallus. It has never been seen to give rise to a sporangium, and probably does not take an active part in the further growth and development of the thallus.

When the germ tube or its branches have become well established in the substratum (fig. 22-25), small refractive globules begin to appear again in the cytoplasm. These globules appear to have a greenish tint, due perhaps to refraction, but as they aggregate in the center of the hyphae and fuse, the resultant larger globules become golden in color. As the globules continue to increase in size, they eventually become peach or apricot in color. The deepening of the color of the globules appears to be due to a concentration of pigment.

STRUCTURE OF THE THALLUS.—The thallus of this chytrid resembles those of *Catenaria*, *Nowakowskiella*, and *Septochytrium* in structure and organization. It exhibits extreme variability in size and shape. Reduced monocentric thalli similar to those of *Karlingia rosea* (Johanson, 1944) are found occasionally, but generally polycentric extensive mycelium-like thalli are developed, which show many of the characteristics noted in other polycentric chytrids. Occasionally empty enlargements are found on the hyphae. They may possibly be equivalent to the spindle organs or "Sammelzellen" characteristic of the genera *Cladochytrium*, *Nowakowskiella*, *Physoderma*, and *Urophlyctis*, but in view of their infrequent occurrence, they are interpreted at present as incipient zoosporangia which failed to mature. At least they are not delimited by septa nor do they become septate like those of *C. replicatum* and *C. tenue*.

The mature sporangia of *C. persicinus* are predominantly intercalary. So far, terminal sporangia have rarely been found. In size and shape there is almost unlimited variation. They may be uteriform, pyriform, ovoid, cylindrical, spherical, elliptical, rectangular, triangular, or irregular. The sporangia are connected by isthmuses which are also vari-

able in size and shape, but the latter are predominantly tubular, unbranched and extensive, when the thalli are growing in grass, corn and onion skin. In cellophane there is no predominance of any particular size and shape, with almost every conceivable variation occurring. In this substratum the isthmuses may be unbranched or branched, short or extensive, cylindrical, elliptical, rectangular, triangular, irregular, or occasionally septate.

SPORANGIAL DEVELOPMENT AND DEHISCENCE.—The sporangia arise as unbranched or branched enlargements of the main and secondary axes. During the early stages of development, the refractive globules flow into the enlargements from the communicating hyphae and rhizoids (fig. 1 D, H). Fusion of refractive globules continues, and at the same time walls are laid down between the incipient sporangium and the remainder of the rhizomycelium.

After a period of seventy-two hours or more, the refringent material begins to disperse again, and gradually disappears as such. The sporangial contents accordingly become finely granular in appearance. During this phase the whole sporangial protoplasm is peach colored, and as in *Karlingia rosea* (Johanson, 1944) this pigmentation remains until the zoospores emerge. It does not disappear with the formation of zoospores as reported by Ward (1939) for the same species. Although the sporangial contents are generally peach or apricot in color, pale yellow sporangia are also found.

By the time the evenly granular stage has been reached, or slightly before, one to several exit tubes develop. They may be comparatively short and broad, or greatly elongated and narrow. It was stated previously (Hanson, 1944) that from one to nine of these exit canals are developed, but sporangia with as many as fourteen exit canals have since been observed. A sporangium with eight exit tubes is shown in figure 1F. Frequently the tubes branch (fig. 1B). The exit canals are at first filled with the same granular cytoplasm as in the sporangium proper (fig. 1E). Soon, however, differentiation occurs at the tip and a series of dehiscence changes begin. The first sign of differentiation is the appearance of a small blister of viscid material at the tip (fig. 29), which gradually enlarges (fig. 30). As it enlarges, the tip of the exit tube becomes dilated (fig. 31-33). At this early stage, two regions of different densities are often distinguishable in the viscid material at the papillar tip (fig. 31-33). The denser region is situated innermost, bounding the protoplasm. The less dense region occupies the most apical portion of the exit tube. As the tip of the exit tube continues to dilate with expanding viscid material, the granular cytoplasm in the tube recedes downward (fig. 34, 35). Exactly how the operculum is formed in the exit tube is not known, but the evidence at hand suggests it develops by condensation at the surface of the granular cytoplasm as it recedes in the tube, in much the same manner as described by Karling in *Nephrochytrium*

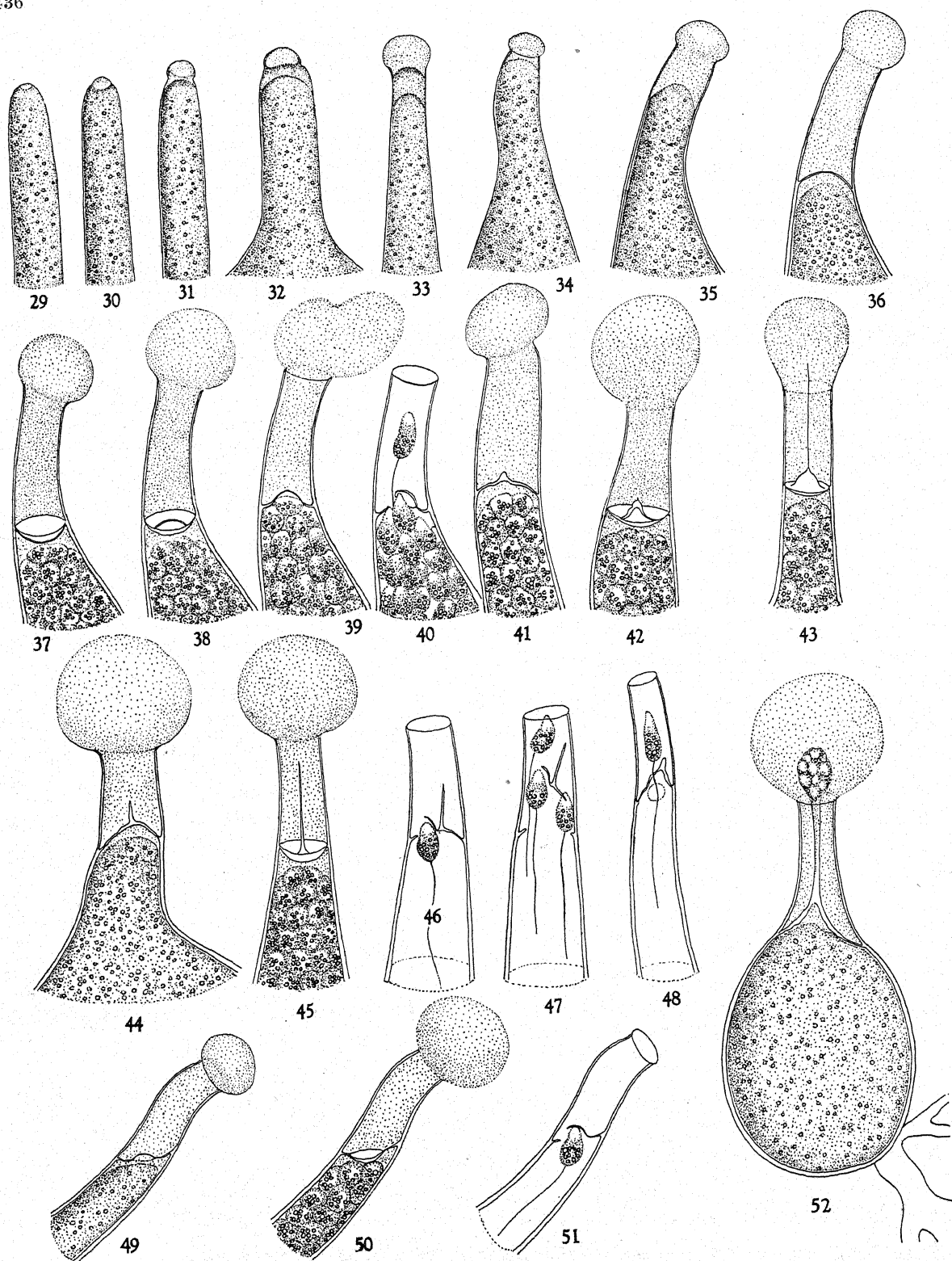


Plate 3. Fig. 29-52, $\times 1680$.—Fig. 29-35. Stages in the formation of the hyaline plug at the tip of the exit canal, with granular cytoplasm gradually receding downward preparatory to the formation of the operculum.—Fig. 36. The operculum has been formed.—Fig. 37. Invagination of the operculum.—Fig. 38. Evagination of the operculum while the dilated tip of exit canal filled with mucilaginous substance is ready to deliquesce.—Fig. 39. Operculum completely evaginated with first zoospores pressing against it. At the tip of the exit tube the mucilaginous substance is dispers-

amazonensis (1944c). Whatever the process may be, the operculum when formed is first convex. In fully mature tubes the spore plasm does not extend completely up to the operculum (fig. 36). The intervening area appears to be filled with a viscid substance which stains magenta with ruthenium red. It is therefore similar in nature to the viscid substance above the operculum which has great affinity for this dye. As the apex of the exit tube continues expanding, the operculum gradually invaginates until it is concave (fig. 37). It retains this shape for from one to four hours and then gradually evaginates and becomes convex again (fig. 38, 39). This evagination of the operculum is more or less synchronous with the deliquescence of the dilated apex of the exit tube and the gradual dispersal of the viscid substance. Spore discharge may begin as soon as all the viscid material has dissolved or may be delayed. In either event the first spores are released under such pressure that the operculum is ripped off and carried out with the spores. The remainder of the spores emerge more slowly, one by one. If the operculum is not torn off (fig. 40), it is raised up by each spore as it passes out. Generally the operculum does not fall all the way back in place before it is struck by the next emerging spore, and when the rate of emergence is rapid, the operculum simply vibrates on its hinge.

As was previously stated, as many as fourteen exit tubes may develop on one sporangium. All undergo simultaneously the same dehiscence changes, but usually only one functions. It is consequently impossible to judge which of the several tubes will function. Accordingly, sporangia with a single exit tube are best suited for the study of discharge. The approximate time at which discharge will occur may be determined by observing the invagination and evagination of the operculum. Evagination is usually completed six hours after invagination began, and as soon as evagination is completed, discharge begins. If the operculum is not immediately torn off, it becomes detached before spore discharge is completed, doubtless because of the great wear and tear to which it is subjected. As a consequence, it is never persistent in a functional exit tube but may be seen in the supernumerary exit tubes which did not function.

As is shown in figures 41 to 48, the opercula may be distinctly apiculate and bear a tenuous hyaline spine (fig. 43-47, 52) as in *Nephrochytrium amazonensis*. Such opercula also may invaginate (fig. 42, 43, 48). As was noted earlier in figures 39 and 40, an apical lid normally operates in the emission of spores, but frequently, when the operculum bears

an apical spine, a small flap lateral to the spine first operates in spore discharge (fig. 46-48). Eventually the whole operculum—lateral flap, apical spine, and all—is torn off and carried out by the spores (fig. 47). This variation suggests that the operculum of *C. persicinus* may sometimes be compound. However, the phenomenon described is noted only when the operculum proper bears a spine. Accordingly, the author at present regards the lateral flap as an abnormality associated with the presence of the spine. Other variations are also to be noted here. The operculum frequently develops at an angle in the exit tube, as is shown in figures 49 to 51.

Typically, the spores emerge one by one in succession and form a temporary but naked group near the orifice of the exit tube. In a few instances, however, the emerged spores were enveloped by a distinct hyaline matrix. The matrix gradually dispersed, but the spores did not become motile. This behavior therefore appears to be the result of premature emission. The matrix enveloping the spores is probably the viscid substance previously noted beneath the operculum (fig. 36-38, 41-45, 49, 50, 52).

The presence of resting spores has not been clearly demonstrated in *C. persicinus*. However, thick-walled cells of the type shown in figures 1K and 1L occasionally occur. They are ovoid, elliptical or somewhat triangular in shape. Their contents consist of one to several large golden refractive globules surrounded by a hyaline homogeneous cytoplasm. These thick-walled cells have never yet been found germinating, and their true significance in the life cycle of *C. persicinus* therefore remains to be determined.

RELATIONSHIPS.—Inasmuch as the presence and method of formation of resting spores have not been definitely established for *C. persicinus*, the exact relationship of this species to other polycentric chytrids is uncertain. As far as is now known, its type of thallus structure and organization is characteristic of that of the family *Cladochytriaceae* as it has recently been interpreted by Whiffen (1944). *Catenomyces persicinus* is accordingly placed in this family for the time being. Within the family our fungus appears to be most closely related to *Septochytrium* and *Nowakowskiella* because of its operculate sporangia. It is to be noted, however, that no well-defined spindle-shaped enlargements have been observed in the tenuous portions of the rhizomycelium like those which occur in some species of *Nowakowskiella*. Furthermore, no trabeculae or septa of the type reported for some species of *Septochytrium* have been seen in our fungus. The only septa so far

ing.—Fig. 40. Raising of the operculum by an emerging amoeboid zoospore.—Fig. 41. An apiculate operculum.—Fig. 42. Partly invaginated apiculate operculum.—Fig. 43. Operculum with delicate apical spine beginning to invaginate.—Fig. 44. An operculum with a short thick spine.—Fig. 45. Complete invagination of a spine-bearing operculum.—Fig. 46. "Compound" operculum with lateral flap.—Fig. 47. Complete detachment of "compound" operculum shown in figure 46.—Fig. 48. Zoospore emerging through a lateral pore from which an operculum was torn by rapidly emerging spores, apiculate region not functioning as an operculum in this instance.—Fig. 49-51. Invagination and dehiscence of an operculum formed at an angle in the exit tube.—Fig. 52. Sporangium with an apiculate operculum. The spine bears at its tip a refractive mass of viscid material.

observed in *C. persicinus* relate to the delimitation of the sporangia from the remainder of the rhizomycelium. Other noticeable differences in our fungus are the lack of apophyses and proliferation of sporangia. In the latter respect *C. persicinus* resembles *Septochytrium* in which proliferation has not yet been observed.

It is obvious that many of the differences cited above are not generically valid for separating *Nowakowskiella*, *Septochytrium*, and *Catenomyces*. In *Nowakowskiella*, for instance, well-defined spindle-shaped enlargements may (*N. elegans*) or may not (*N. profusa*) be present, while in *Septochytrium* trabeculae or septa may be lacking (*S. macrosporum*; Karling, 1942) in the tenuous filaments. Furthermore, deliquescence of the tip of the exit tube and the formation of endo-opercula somewhat similar to those of *C. persicinus* also occur in *N. granulata* and *N. macrospora* (Karling, 1944a, 1945). This type of sporangial dehiscence has also been observed in *Karlingia rosea* (Johanson, 1944) and *Nephrochytrium amazonensis* (Karling, 1944c), monocentric chytrids of the family Rhizidiaceae. Obviously, this character also is generically invalid. Accordingly, a careful analysis of the similarities and differences noted above raises the question of whether *Septochytrium* and *Catenomyces* should be merged with the large polycentric operculate genus, *Nowakowskiella*. As far as present knowledge goes, there appear to be no substantial grounds for separating these genera. However, since *C. persicinus* is not completely known and may possibly show other relationships, it is retained for the present in *Catenomyces*.

Mention has already been made of the striking similarity of *Catenomyces* to *Catenaria*. However, the presence of an operculum in the former genus precludes very close relationship between the two. Nevertheless, the presence of tubular isthmuses between the sporangia and numerous fine sterile rhizoids suggests that *Catenomyces* may be the oper-

culate counterpart of *Catenaria* which has arisen by parallelism in evolution and development. It is to be noted, further, that the zoospores of *C. persicinus* include a large number of small globules like those of *Catenaria anguillulae* and usually emerge and disperse in a similar manner. This similarity, however, does not in itself indicate close relationship, but if, in addition, the resting spores of *Catenomyces* prove to be similar in structure and formation to those described by Couch (1945) for *Catenaria* species, then our fungus must be transferred to the Blastocladales. In this event, *Catenomyces* would be the first operculate member of the Blastocladales.

The only other operculate polycentric genus to which *Catenomyces* may possibly be related is *Megachytrium* (Sparrow, 1931, 1933). This genus is characterized by tubular, branched, coarse mycelioid occasionally septate thallus, the extremities of which do not run out to fine threads. Its thallus is accordingly non-rhizoidal and differs fundamentally in this respect from that of *Catenomyces*.

SUMMARY

Catenomyces persicinus occurred as a saprophyte in cellophane, bleached corn and grass leaves, and dried onion skin suspended in samples of water from Quaker Brook near Sharon, Connecticut. The fungus is a pigmented "endo-operculate" polycentric chytrid belonging to the family Cladochytriaceae, and its similarity and differences with other members of the family have been pointed out. The occurrence of true resting spores has to date not been determined, and the exact relationship of this fungus to other polycentric chytrids is consequently uncertain. When resting spores and their germination are definitely known, the inclusion of the new fungus in the genus *Nowakowskiella* may be possible.

DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY,
NEW YORK 27, NEW YORK

LITERATURE CITED

- AJELLO, L. 1942. *Polychytrium*: A new cladochytriaceous genus. *Mycologia* 34: 442-451.
- COUCH, J. N. 1941. The structure and action of the cilia in some aquatic Phycomycetes. *Amer. Jour. Bot.* 28: 704-713.
- . 1945. Observations on the genus *Catenaria*. *Mycologia* 37: 163-193.
- , AND A. J. WHIFFEN. 1942. Observations in the genus *Blastocladiella*. *Amer. Jour. Bot.* 29: 582-591.
- HANSON, A. M. 1944. Three new saprophytic chytrids. *Torreyia* 44: 30-33.
- HILLEGAS, A. B. 1940. The cytology of *Endochytrium operculatum* (de Wildeman) Karling in relation to its development and organization. *Bull. Torrey Bot. Club* 67: 1-32.
- JOHANSON, A. E. 1944. An endo-operculate chytridiaceous fungus: *Karlingia rosea* gen. nov. *Amer. Jour. Bot.* 31: 397-404.
- KARLING, J. S. 1934. A saprophytic species of *Catenaria* isolated from roots of *Panicum variegatum*. *Mycologia* 26: 528-543.
- . 1937. The cytology of the Chytridiales with special reference to *Cladochytrium replicatum*. *Mem. Torrey Bot. Club* 19(1): 3-92.
- . 1942. A new chytrid with giant zoospores: *Septochytrium macrosporum* sp. nov. *Amer. Jour. Bot.* 29: 616-622.
- . 1944a. Brazilian chytrids. I. Species of *Nowakowskiella*. *Bull. Torrey Bot. Club* 71: 374-389.
- . 1944b. Brazilian chytrids. II. New species of *Rhizidium*. *Amer. Jour. Bot.* 31: 254-261.
- . 1944c. Brazilian chytrids. III. *Nephrochytrium amazonensis*. *Mycologia* 36: 351-357.
- . 1945. Brazilian chytrids. V. *Nowakowskiella macrospora* n. sp., and other polycentric species. *Amer. Jour. Bot.* 32: 29-35.
- SPARROW, F. K., JR. 1931. A note on a new chytridiaceous fungus parasitic in *Elodea*. *Occas. Papers Boston Soc. Nat. Hist.* 8: 9-10.
- . 1933. Observations on operculate chytridiaceous fungi collected in the vicinity of Ithaca, N. Y. *Amer. Jour. Bot.* 20: 63-77.
- WARD, M. W. 1939. Observations of *Rhizophlyctis rosea*. *Jour. Elisha Mitchell Sci. Soc.* 55: 353-360.
- WHIFFEN, A. J. 1944. A discussion of taxonomic criteria in the Chytridiales. *Farlowia* 1: 583-597.

THE RELATION OF GROWTH TO SIZE IN CUCURBIT FRUITS¹

Edmund W. Sinnott

AN UNDERSTANDING of the genetic basis of inherited differences among organisms must depend on a knowledge of how these differences arise during development. Characters of size, although notably difficult to analyze genetically, have a marked advantage for developmental studies since they can be examined throughout most of the period from inception to maturity, and since many developmental differences among them can be described in simple quantitative terms. It is possible, for example, to determine whether a given inherited size difference is due to a difference in rate or in duration of growth, and thus to state the problem of its genetic control more nearly in physiological terms.

The fruits of the Cucurbitaceae and particularly of *Cucurbita Pepo* offer good material for such studies. Size differences among races of this species are very great, some of the largest pumpkins bearing fruits a thousand times the volume of the smallest gourds. The ovaries and fruits are easily accessible and their dimensions can be measured from a very early stage. They are determinate structures, each an organic entity with a developmental cycle of its own which is comparable to that of an individual organism. In many cases pure lines, essentially homozygous, have been established by inbreeding. The purpose of the present paper is to compare fruit growth, from early ovary size to maturity, in a number of races differing markedly in inherited fruit size, and thus to provide evidence helpful in a developmental analysis of such differences.

Relatively few studies have been made of the growth of fruits. Anderson (1895) brought a young and growing pumpkin fruit into the laboratory, still attached to its vine, and weighed it for a period of 47 days. Growth showed a sigmoid curve and the data were later analyzed by Robertson (1923). Gustafson (1926) studied the growth of a number of cucurbit fruits, with results much like Anderson's. Growth curves have been determined for a few other fruits, such as tomatoes, apples, peaches, and orchids. The growth of leaves, also determinate plant structures, has been measured by a number of workers, notably by Weizsacker (1938) in tobacco. In all cases the basic growth program is that of a period of accelerating growth followed by a fall in rate until maturity, which produces, when plotted arithmetically against time, the familiar sigmoid curve. Little work seems to have been done, however, on the comparative growth behavior of large and small types in the same species.

MATERIALS AND METHODS.—A number of inbred lines of *Cucurbita Pepo* were studied. The five most thoroughly analyzed were *SRC*, a white spheroidal

line with a fruit volume at maturity of about 50 cc.; *TA*, a white, ovoid type of about 40 cc.; *SP*, a green and yellow "spoon" gourd of about 70 cc.; *CF*, a large yellow pumpkin of about 7,000 cc.; and *M35*, another pumpkin type of about the same volume. Also studied were lines *MT*, an elongate green pumpkin with a fruit volume of 4000 cc.; *O*, a round "orange" gourd of about 175 cc., and *P*, a small "sugar" pumpkin of about 800 cc. These are all vine or "runner" types, but two "bush" races were also studied: *103*, a nearly spherical white line, with a fruit volume of about 700 cc., and *STN*, a yellow "straight-neck" summer squash of about 800 cc. Aside from these lines of *Cucurbita Pepo*, two from *Lagenaria vulgaris* were studied: line *TR*, a pear-shaped type with a fruit volume of about 650 cc., and *MB*, a small "bottle" gourd, of about 60 cc. A few fruits from plants of watermelon grown from commercial seed were also measured.

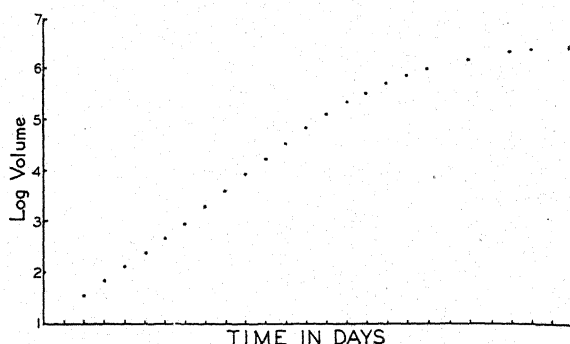


Fig. 1. Plot of the logarithms of the daily volume measurements of a typical fruit belonging to line *M35*.

In a number of cases, fruit growth on plants derived from crosses between large and small races was studied and compared with that of the parent types. Twenty-nine plants of one *F*₂ population were also analyzed.

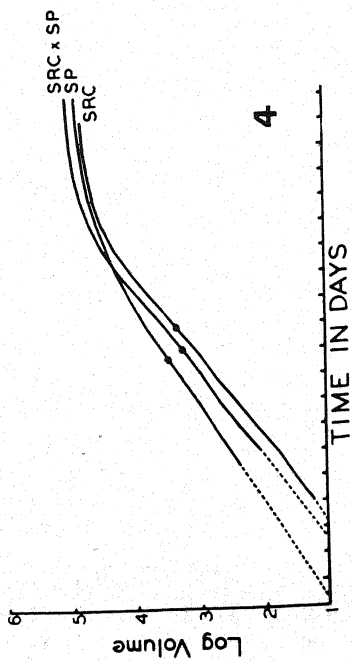
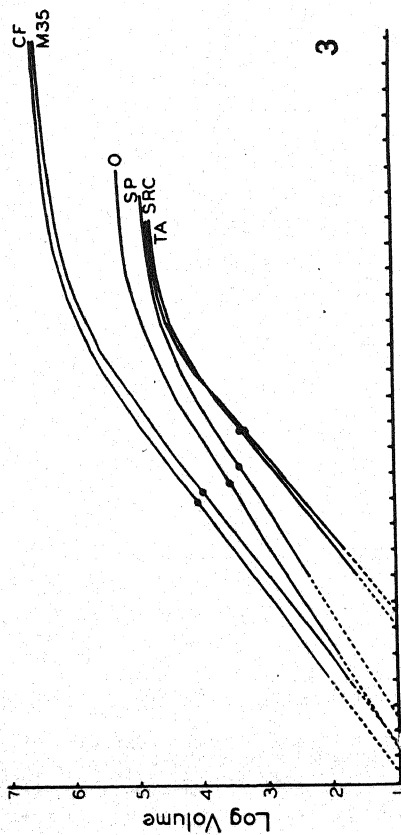
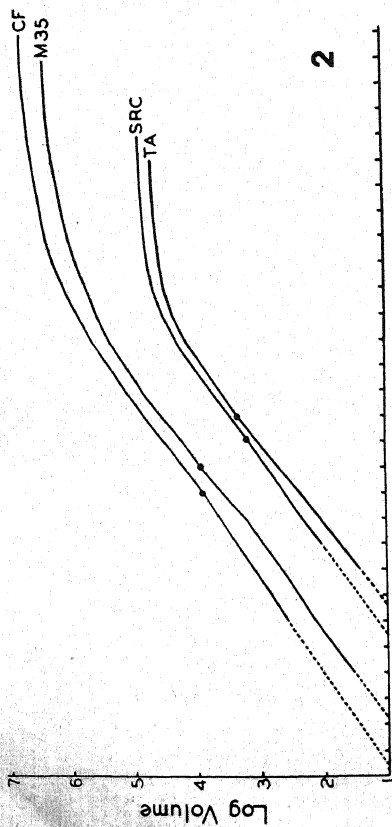
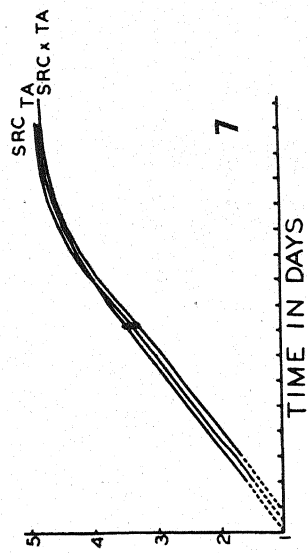
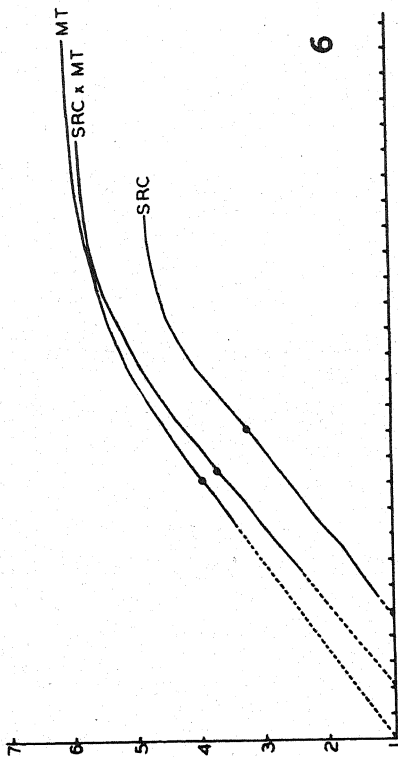
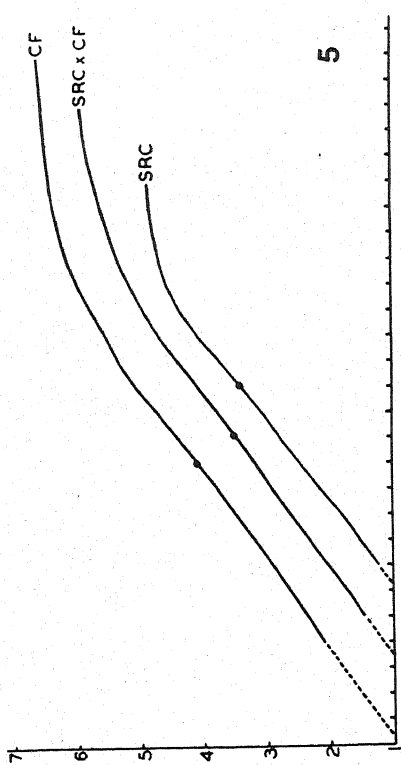
In lines *SRC*, *TA* and *TR*, the growth of fruits on diploid plants was compared with that of tetraploids derived from the same lines.

Data were gathered from cultures made in six seasons, 1937, 1938, 1939, 1940, 1941, and 1944. These were grown in three localities: Woodbury and New Haven, Connecticut; and Falmouth, Massachusetts. So far as possible, cultural conditions were kept the same in all places. Seeds were planted outdoors between May 15 and June 1 and most measurements made during the last week of July and the first three weeks of August. During two seasons, temperature and other weather conditions were recorded.

Measurements of small ovaries and young fruits were made with steel calipers registering (with vernier) to .1 mm. Ovaries as small as 2 mm. in diameter could be measured with accuracy if care was

¹ Received for publication February 6, 1945.

The author wishes to express his thanks to Edmund W. Sinnott, Jr. and to Mildred Sinnott Hill for their efficient assistance during the course of this investigation.



used, but in most cases records were not begun until the ovary had reached 3 mm. or more. Most measurements were made daily, though occasionally a two-day interval occurred between them. Maximum equatorial diameter was always measured and in most series length or polar diameter, as well, taken from the base of the ovary to the insertion of the calyx. Volume was computed as the square of the diameter multiplied by length and (to correct for the generally spheroidal form) divided by two. In cases where diameter alone was measured, length was supplied from previously determined relations of length to diameter at various sizes. Such computations of volume are by no means entirely accurate but they give good approximations to absolute volume and for purposes of rate determination are perfectly satisfactory.

The day of flowering was always recorded, as was the day when significant increase in size no longer occurred.

Ovaries or fruits which became scarred or were diseased or otherwise abnormal were discarded.

RESULTS—The smallest ovary volumes which could be measured with accuracy were about 10 cubic millimeters. This increased during growth to a final volume of about 40,000 cu.mm. in the small-fruited races and to over 10,000,000 cu.mm. in the largest fruits. In the former this growth was accomplished in about 16 to 18 days but in the latter it usually required more than 30 days.

When ovary volume is plotted against time, a typical sigmoid curve results, resembling those found by previous workers. Since the measurements here recorded, however, go back to such a very small size, as compared to that of the mature fruit, the changes in volume can be shown graphically in a much more complete fashion if the logarithms of volume rather than the volumes themselves are plotted, and this method has therefore been used in the present paper. The daily volume measurements of a typical fruit plotted in this way are shown in figure 1. These points fall along an essentially straight line during the early part of the growth curve but this line later flattens out gradually until growth ceases.

During the period when the line is straight, growth is evidently taking place at a constant exponential rate, the fruit growing as at compound interest. The slope of the line measures the rate of growth. The actual rate for this portion of the curve, or any part of it, can be readily computed from Blackman's (1919) equation,

$$r = \frac{\log w_1 - \log w_0}{t \log e}$$

Figs. 2-7. In all figures the logarithm of ovary volume in cubic millimeters is plotted against time in days. In each case the first portion of the line, essentially straight, is carried back (broken line) to a volume of 10 cu.mm. from which, as an arbitrary origin, the duration of growth can be measured. The time of flowering is shown by a circle on the line. Each line is carried to the point at which growth ceases.—Fig. 2. Curves of four typical individual fruits of the same year (1939), from two large-fruited lines and two small ones.—Fig. 3. Average curves for six lines for the year 1939.—Figs. 4-7. Curves for four pairs of lines and, in each case, for the F_1 from a cross between them grown in the same year.

where w_0 is the volume at the beginning of the period, w_1 the volume at a given time t afterward, and e the base of the natural logarithms. In most fruits measured the rate was found to be from 60 per cent to 70 per cent per day, which, since it is compounded continuously, means that the ovary approximately doubles its volume daily. The end of exponential growth—the point where the line ceases to be straight and begins to flatten out—can usually be determined rather exactly.

To compare the relative duration of growth in different types it is necessary to find a comparable starting point for all of them. Since growth in very early stages could not be measured, an arbitrary ovary volume, 10 cubic mm., was selected as a base from which to calculate growth duration in all types. In most cases measurement began at a somewhat larger size than this and since the first part of the curve is essentially straight, it seems legitimate to extrapolate the curve back to the arbitrary volume, the logarithm of 10 cubic mm. In all figures such extrapolation is indicated by a broken line. From the point where this meets the arbitrary origin, duration of growth in days has been measured. The curves in figures 2-7 are drawn through such series of points as are given in figure 1. None of the curves are smoothed. The day of flowering is indicated by a solid circle in the line. The end of the line is on the day where significant increase in volume ceased.

The growth of fruits of large-fruited and small-fruited lines can be compared by studying their growth curves. In figure 2 are shown the curves of four individual fruits, two large and two small. In figure 3 are the average curves for all individuals studied in six lines for the year 1939. Each of these averages was determined by taking a comparable point—the day when growth rate began appreciably to decrease—on the curve of each individual fruit of that line measured, and finding the average ovary volume for the line at that point. The average volumes for successive days before and after this point were then determined and the curve drawn through them. Practically identical curves result if the other good comparable point—the day of flowering—is used instead.

From these curves it is possible to determine a number of facts as to fruit growth in each line which may then serve as a basis for comparing the lines with each other. These facts are: the duration of the entire growth period in days, from a volume of 10 cu.mm.; the duration of each comparable part of it—from the origin to flowering, from flowering to the break in the curve (the end of exponential growth)

TABLE 1. Data for duration and rate of growth, size of ovary at flowering, and final fruit size.

Line	Year	No. of fruits	Duration in Days			Rate	Log ovary volume at flowering	Log fruit volume at final size
			Total	Origin to flowering	Flowering to final size			
SRC	1937	21	18.5	8.5	10.0	.602 ± .007	3.277 ± .034	4.892 ± .031
	1938	18	16.5	9.5	7.0	.680 ± .011	3.190 ± .037	4.590 ± .241
	1939	3	16.0	7.5	8.5	.701 ± .011	3.258 ± .031	4.803 ± .059
	1944	7	17.5	8.0	9.5	.666 ± .012	3.330 ± .045	4.770 ± .034
	Aver.		17.13	8.38	8.75	.648 ± .007	3.253 ± .021	4.754 ± .023
TA	1937	1	22.5	7.5	15.0	.606	3.399	5.234
	1938	27	17.0	7.5	9.5	.688 ± .011	3.180 ± .037	4.510 ± .048
	1939	8	16.0	8.0	8.0	.682 ± .013	3.403 ± .025	4.812 ± .018
	1944	3	19.0	9.0	10.0	.606 ± .037	3.623 ± .091	4.782 ± .067
	Aver.		18.63	8.0	10.33	.679 ± .009	3.269 ± .036	4.615 ± .043
CF	1937	5	31.0	12.0	19.0	.566 ± .010	3.932 ± .045	6.715 ± .037
	1938	4	30.5	13.5	17.0	.611 ± .012	3.950 ± .096	6.570 ± .102
	1939	7	28.0	10.5	17.5	.650 ± .010	3.971 ± .054	6.612 ± .080
	1944	6	32.5	11.5	21.0	.619 ± .023	4.177 ± .046	6.945 ± .111
	Aver.		30.5	11.88	18.62	.615 ± .010	4.014 ± .032	6.692 ± .048
M35	1937	2	36.5	12.5	24.0	.531 ± .001	4.064 ± .406	7.172 ± .128
	1938	1	33.0	13.0	20.0	.574	3.824	5.720
	1939	4	27.5	9.5	18.0	.669 ± .006	3.948 ± .042	6.559 ± .088
	1944	2	32.5	12.0	20.5	.554 ± .034	4.158 ± .037	6.700 ± .192
	Aver.		32.38	11.75	20.33	.602 ± .022	4.007 ± .050	6.630 ± .145
Total	1937		27.5	10.0	17.5	.576 ± .018	3.668 ± .194	6.003 ± .453
	1938		21.5	10.5	11.0	.638 ± .028	3.536 ± .204	5.350 ± .492
	1939		21.0	8.5	12.5	.676 ± .011	3.645 ± .184	5.697 ± .513
	1944		25.0	10.0	15.0	.611 ± .023	3.822 ± .204	5.799 ± .593
Sp	1939	6	20.0	9.5	10.5	.540 ± .017	3.437 ± .097	4.893 ± .041
	1944	2	21.0	9.5	11.5	.455 ± .029	3.211 ± .179	4.698 ± .167
	Aver.		20.5	9.5	11.0	.519 ± .019	3.372 ± .090	4.850 ± .052
O	1939	3	22.0	9.0	13.0	.549 ± .004	3.540 ± .019	5.171 ± .040
	1944	3	25.5	12.5	13.0	.470 ± .040	3.860 ± .086	5.257 ± .141
	Aver.		23.75	10.75	13.0	.510 ± .031	3.700 ± .074	5.214 ± .067
MT	1937	2	28.5	13.5	15.0	.476 ± .002	4.493 ± .259	6.324 ± .027
	1939	2	27.5	8.5	19.0	.592 ± .021	3.942	6.425 ± .139
	Aver.		28.0	11.0	17.0	.534 ± .035	4.309 ± .236	6.225 ± .080
P	1937	3	25.0	14.5	10.5	.635 ± .011	4.010 ± .006	5.899 ± .024
103	1937	2	25.0	14.5	10.5	.520 ± .019	4.433 ± .018	5.897 ± .026
	1939	1	26.0	14.0	12.0	.485	4.107	5.709
	Aver.		25.5	14.25	11.25	.508 ± .010	4.324 ± .106	5.834 ± .063
STN	1939	2	23.0	10.5	12.5	.561 ± .023	3.801 ± .046	5.896 ± .026
TR	1941	10	23.0	8.0	15.0	.515 ± .012	2.853 ± .084	5.690 ± .099
MB	1939	2	17.5765 ± .018	4.801 ± .179
WM	1937	2	20.5	7.0	13.5	.727 ± .130	3.908 ± .389	6.341 ± .519
	1938	3	27.5	6.0	21.5	.684 ± .085	2.860 ± .078	6.170 ± .161
	Aver.		24.0	6.5	17.5	.701 ± .055	3.279 ± .289	6.240 ± .198

and from this point to final size; the rate of growth during the exponential period; the ovary volume at flowering and the fruit volume at final size.

Determinations of six of these values for the six lines shown in figure 3, together with seven others less intensively studied, are presented in table 1. The logarithms of ovary and fruit volumes, rather than the volumes themselves, are given. Errors are standard errors.

A comparison, on the basis of data from figures 2

and 3 and table 1, between lines unlike in mature fruit size brings out a number of facts with regard to fruit growth in these lines.

First, in all lines growth consists of two phases, an initial one in which there is a constant exponential rate, followed by a phase of continually decreasing rate until growth ceases.

Second, there is no relation between growth rate, either during the exponential period or later, and final fruit size. One of the small lines (SP) has a

very low rate and another (*MB*) the highest of all. The very large lines are intermediate.

Third, as a corollary of this, the larger a fruit is at maturity, the longer it takes to grow. Not only the whole period of growth but each portion of it—from the beginning to flowering, from flowering to the "break" and from thence to final size—is of longer duration in a large-fruited type than in a small-fruited one. The growth curve of the large type, when expressed logarithmically, is essentially a replica of that of a small one of the same rate save that it is enlarged in all its parts. It follows that in large-fruited types each comparable stage during growth is longer and the amount of growth between one stage and the next is greater than in the smaller ones.

Fourth, there are differences in growth rate between the various lines. *TA*, for example, is consistently higher than *M35*; *MB* is very high, and *SP*, *O* and *103* are markedly low. The absolute differences between these rates are not very great, but the differences exist and in many cases are definitely significant, especially when data from the same season are used. There seems to be no relation between growth rate and any other character of the line.

Fifth, environmental factors, insofar as these are reflected in seasonal differences, markedly affect rate. This is shown best by a comparison between the four seasons for the four lines (*SRC*, *TA*, *CF* and *M35*) most intensively studied. The average rates ranged from .576 in 1937 to .676 in 1939. To separate the differences due to the years from those due to the lines, an analysis of variance was made for which the author is indebted to his colleague, Dr. C. I. Bliss, and to Dr. W. G. Cochrane. Owing to the wide variation in the numbers of individuals in the different races and years, it was difficult to determine variance in the usual way. The method here used is based upon that of fitting constants, described by Yates (1934). From the results, set forth in table 2, it can be shown that the differences between lines are significant but those between years are even more highly so.

Sixth, there is an inverse relation between growth rate and growth duration in the various seasons. When rate is high, final size is attained more rapidly than when it is lower. This is also evident not only in the seasonal averages but in fast-growing and slowly-growing plants of the same line. There is some evidence of the same sort of compensation between lines which differ in rate, the more slowly growing ones, such as *SP*, having a longer growth period than those of about the same final size but faster growth.

A comparison between pure types and first generation hybrids between them shows a considerable diversity in behavior. Where a large-fruited race like *CF* is crossed with a small one like *SRC* (fig. 5) the F_1 was found to be intermediate in fruit size and not far from the geometric mean between the two

parents. In one year the F_1 was about equal to the larger parent at flowering, but reached this stage in 10 days rather than 12, growing at a faster rate. It was outdistanced by *CF*, however, in the latter part of the growth cycle, since its growth fell off more rapidly and ceased four days earlier. In another year (fig. 5) the F_1 was about the size of the smaller parent at flowering but its growth continued much longer, though at all stages it was definitely behind

TABLE 2. Analysis of variance in growth rates for four lines for four years.

	Degrees of freedom	Mean squares	F
Years	3	.04847	18.57
Lines (eliminating years)	3	.01773	6.79
Interactions	9	.00261	1.00 1.43

the larger parent. Different results were obtained, however, when *SRC* was crossed with another large-fruited line, *MT*. Here the F_1 was not far behind the larger parent at maturity, having a higher growth rate but a shorter total growth period (fig. 6).

In crosses between two lines differing but little in final size or in rate, the F_1 (fig. 7) was intermediate throughout its growth, though somewhat nearer the larger parent at maturity.

When two lines, *SRC* and *SP*, not far apart at final size but differing in growth rate, were crossed (fig. 4) the F_1 showed a rate essentially like that of the faster parent, *SRC*, and also excelled the latter in duration of growth, so that in this case the F_1 was larger than either parent, though not markedly so.

An F_2 generation of 29 plants grown from this last cross was analyzed and compared with its F_1 and the parental types. The data are shown in table 3. As to rate, the variability of the F_2 is significantly higher than that of the F_1 and of one of the parents. In final fruit size, however, F_2 variability is not significantly greater than in F_1 .

In three lines, *TA*, *SRC* and *TR*, the diploid race was compared as to fruit development with a tetraploid one derived from it by colchicine treatment. The mature fruit was somewhat larger in the tetraploid in *TR*, somewhat smaller in *TA*, and about the same size as the diploid in *SRC*. Growth rates were not greatly different. In *TR* diploid it was $.515 \pm .012$ and in *TR* tetraploid, $.452 \pm .017$. In *TA* the diploid rate was $.535 \pm .019$ and the tetraploid $.606 \pm .014$. In *SRC* the diploid rate was $.550 \pm .040$ and the tetraploid $.552 \pm .013$. Thus in *TR* the diploid rate was slightly but significantly higher; in *TA* it was slightly but significantly lower; and in *SRC* the two types were practically identical in rate.

DISCUSSION.—The genetic control of differences

TABLE 3. Data for parents, F_1 and F_2 of a cross between a rapidly growing line and a slowly growing one.

Line	Year	No. of fruits	Duration in Days			Rate	Standard deviation	Log ovary volume at flowering	Standard deviation	Log fruit volume at final size	Standard deviation
			Total	Origin to flowering	Flowering to final size						
SRC	1939	3	16.0	7.5	8.5	.701 \pm .011	.019 \pm .008	3.258 \pm .031	.053 \pm .022	4.803 \pm .059	.102 \pm .042
Sp	1939	6	20.0	9.5	10.5	.540 \pm .017	.041 \pm .012	3.437 \pm .097	.218 \pm .069	4.893 \pm .041	.109 \pm .029
F_1 SRC \times Sp	1939	3	17.5	7.5	10.0	.700 \pm .013	.022 \pm .009	3.242 \pm .123	.212 \pm .087	5.025 \pm .066	.114 \pm .047
F_2 SRC \times Sp	1940	29	19.0	8.0	11.0	.660 \pm .013	.070 \pm .009	3.362 \pm .024	.122 \pm .016	5.044 \pm .035	.188 \pm .025

in fruit size evidently operates primarily by determining the duration of the growth period. The small-fruited types, although growing as fast as the large ones, cease growth much earlier. This suggests that there is available to each of them a smaller amount of some essential substance or substances, produced under the control of genetic factors, which might determine size in this way. These substances cannot be the elaborated materials from which the fruit is built, since such materials are present in abundance. In some lines there are made from them many small fruits, but in others a few much larger ones. The difficult problem is to find why the amount allocated to each fruit in one line is so different from that in another line.

This control may more hopefully be sought among physiologically active substances like the vitamins and hormones, which have been found to affect growth powerfully even when present in extremely small amounts. It would be useful to know the concentration of such substances in the various developmental stages of fruits which differ in final size. A beginning on this problem has been made by Dr. Katherine Wilson in this laboratory (paper unpublished). She has found that three B vitamins tested become progressively reduced in concentration during the period of exponential growth. When the concentration is reduced to a certain level, which is essentially the same in all types, the rate of growth begins to decrease. This reduction in vitamin content proceeds much more rapidly in small-fruited than in large-fruited races, so that in the former the critical level is reached at a much smaller ovary size than in the latter. Thus there seems to be a relation between the concentration of these three vitamins and the duration of growth. Doubtless other growth substances are also involved. Whether genetic factors control the rate at which such substances are produced, the rate at which they are broken down, or the rate at which they enter the growing fruit, is not known. It seems probable that in a fruit which is destined to be large the necessary amounts of such substances are maintained for a longer time than in one which will be small. The relation between growth substances and inherited organ size offers a hopeful field for a study of the chemical basis of gene action.

It is noteworthy that even when the growth rate is markedly different, as it sometimes is in different seasons, the final fruit size attained is essentially the same. Lower rate of growth is compensated by longer duration in all phases. This suggests that there may be a relation between the absolute amount of essential substances available and total growth. This total amount evidently has a very definite limit, for it has proved impossible to make a small-fruited type bear larger fruits by the most favorable cultural practices. A few attempts to inject physiologically active substances into developing ovaries had no effect but offers a promising method of attack on the problem.

The fact that in the larger fruits all parts of the growth curve are of longer duration is evidently in conflict with Robertson's hypothesis that the growth curve is similar to that of a monomolecular autocatalytic reaction, each stage of which can be determined from the equation

$$\log \frac{A}{A - X} = k(t_1 - t)$$

in which X is the size at any time; t_1 , A the final size; t the time when half the final size is reached; and k a constant relating to growth rate. Rate at any time is proportional to the amount yet to grow, and thus decreases steadily from the beginning of development. If the logarithm of size is plotted against time, as has been done in the present paper, the curve will therefore be convex throughout its course. However, where final size is vastly greater than initial size (as in the material here analyzed) the value of $A - X$ is for a long time subject to such slight change per unit of time as to be essentially constant. Thus a growth curve plotted logarithmically from values derived by Robertson's equation will follow for a considerable time (if the rate is fairly high) an essentially straight line. As the structure begins to increase markedly in size, this nearly straight line changes to a gradually flattening curve.

The growth curve of a large fruit, such as that of line CF , fits rather well one calculated from Robertson's equation, in which k is taken as r (derived from the straight part of the line) multiplied by $\log e$. If the growth of a much smaller fruit, like TA , is calculated in the same way, however, and with the same value for k , it will be markedly different from the curve actually found for this line. If Robertson's equation is followed, the number of days between the point where the fruit is half grown and the point where final size is attained must be the same for fruits of all sizes (with the same growth rate). Actually, however, the time between half size and final size is much shorter in the small fruited types than in the large ones. Therefore, although Robertson's formula gives a fair approximation to some of the curves here obtained, the correspondence with others is far from close.

So many factors are involved that it seems unlikely that any single equation will fit all these curves exactly. Early growth is strictly exponential, which would be expected in a totally embryonic system. It is noteworthy that this is the period, in these fruits, in which cell division is actively taking place (Sinnott, 1939). What causes growth to diminish in rate and finally cease is the most difficult part of the problem.

There is little evidence of heterosis in the present material. In only one case (fig. 4) did the F_1 exceed both parents in final size. Here its growth rate was like that of the faster growing parent, which it exceeded because of a somewhat longer growth period. In most cases the F_1 was intermediate between the

two parents. Sometimes it was a little faster than either in early growth but much behind the larger parent in duration. Sometimes its duration was great enough to put it close to the larger parent. These results confirm East's conclusion that heterosis is little evident in organs of determinate growth.

Such evidence as there is suggests that there are inherited differences in growth rate. Some races are significantly slower in growth than others; and in the single F_2 generation analyzed, from a cross between two races differing in rate, there is a significant increase in variability of rate over the pure types and the F_1 , indicating segregation of rate factors. Final fruit size, in which the parents were not very unlike, is not significantly more variable in the F_2 . Differences in rate are clearly minor elements in the determination of size.

Despite a considerable body of evidence from other plants that tetraploids and diploids differ in growth, little consistent information is given by the present study. Here again, conditions may be different in organs of limited growth, like the fruit, from those in the plant as a whole, where growth is essentially indeterminate.

SUMMARY

The volume of ovary and fruit was measured daily in a number of lines of cucurbits, chiefly *Cucurbita Pepo*, which differ markedly in mature fruit size. These ranged from small gourds of about 40 cc. to large pumpkins of about 7000 cc.

Growth was studied from these measurements and from curves of the logarithm of volume plotted against time.

Growth in all cases consists of an initial phase of constant exponential rate followed by one of gradual decrease.

Final fruit size has little relation to rate of growth but is determined chiefly by its duration. In large-fruited races each portion of the growth cycle is of longer duration than in small-fruited ones.

There are slight inherited differences in growth rate between the various lines.

Environmental factors, as reflected in the results of six seasons, affect growth rate and duration, but fruit size much less. When growth rate is relatively high, final size is attained more rapidly than when the rate is lower, but the size itself is essentially the same.

There is little evidence of heterosis in fruit size, the F_1 fruits in most cases being not far from the geometric mean of their parent types. In three lines studied there is no constant difference in fruit growth between diploid and tetraploid races.

Robertson's equation for growth fits the large-fruited types fairly well but not the small ones.

It is suggested that genetic factors for fruit size may operate by controlling the production or destruction of physiologically active substances necessary for fruit growth.

YALE UNIVERSITY,
NEW HAVEN, CONNECTICUT

LITERATURE CITED

- ANDERSON, A. P. 1895. The grand period of growth in a fruit of *Cucurbita Pepo* determined by weight. Minnesota Bot. Studies 1:238-279.
- BLACKMAN, V. H. 1919. The compound interest law and plant growth. Annals Bot. 33:353-360.
- GUSTAFSON, F. G. 1926. Growth studies on fruits. Plant Physiol. 1:265-272.
- ROBERTSON, T. B. 1923. The chemical basis of growth and senescence. 389 p. Philadelphia.
- SINNOTT, E. W. 1939. A developmental analysis of the relation between cell size and fruit size in cucurbits. Amer. Jour. Bot. 26:179-189.
- WEIZSÄCKER, W. 1938. Beitrag zur Studium des Wachstumsvorganges bei Pflanzen. Arch. Entwickl. Org. 137:34-56.
- WILSON, KATHERINE S. 1944. Vitamin patterns in the development of cucurbit fruits. Thesis, Department of Botany, Yale University.
- YATES, F. 1934. The analysis of multiple classifications with unequal numbers in the different classes. Jour. Amer. Statistical Assoc. 29:51-66.

ABCDEFGHIJKLMNQRSTUUVWXYZ

1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



ABCDEFGHIJKLMNQRSTUUVWXYZ

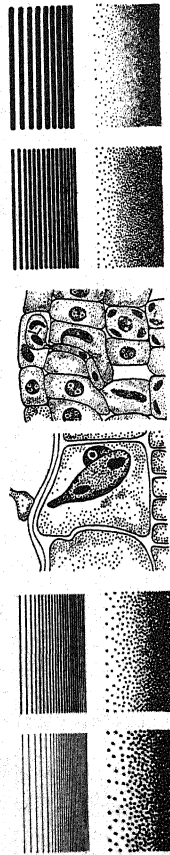
1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



ABCDEFGHIJKLMNQRSTUUVWXYZ

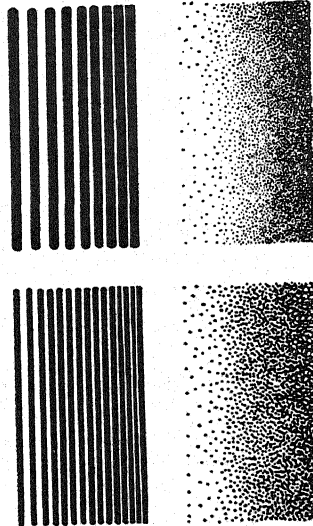
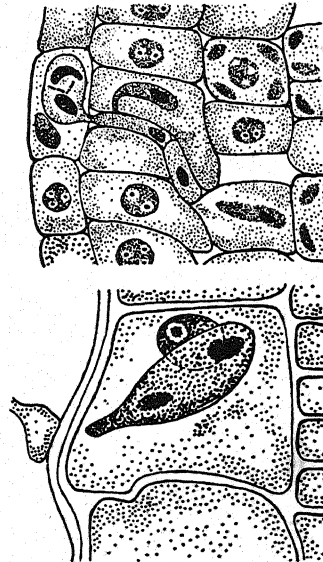
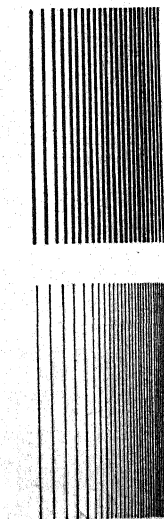
1234567890

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ

ABCDEFGHIJKLMNQRSTUUVWXYZ



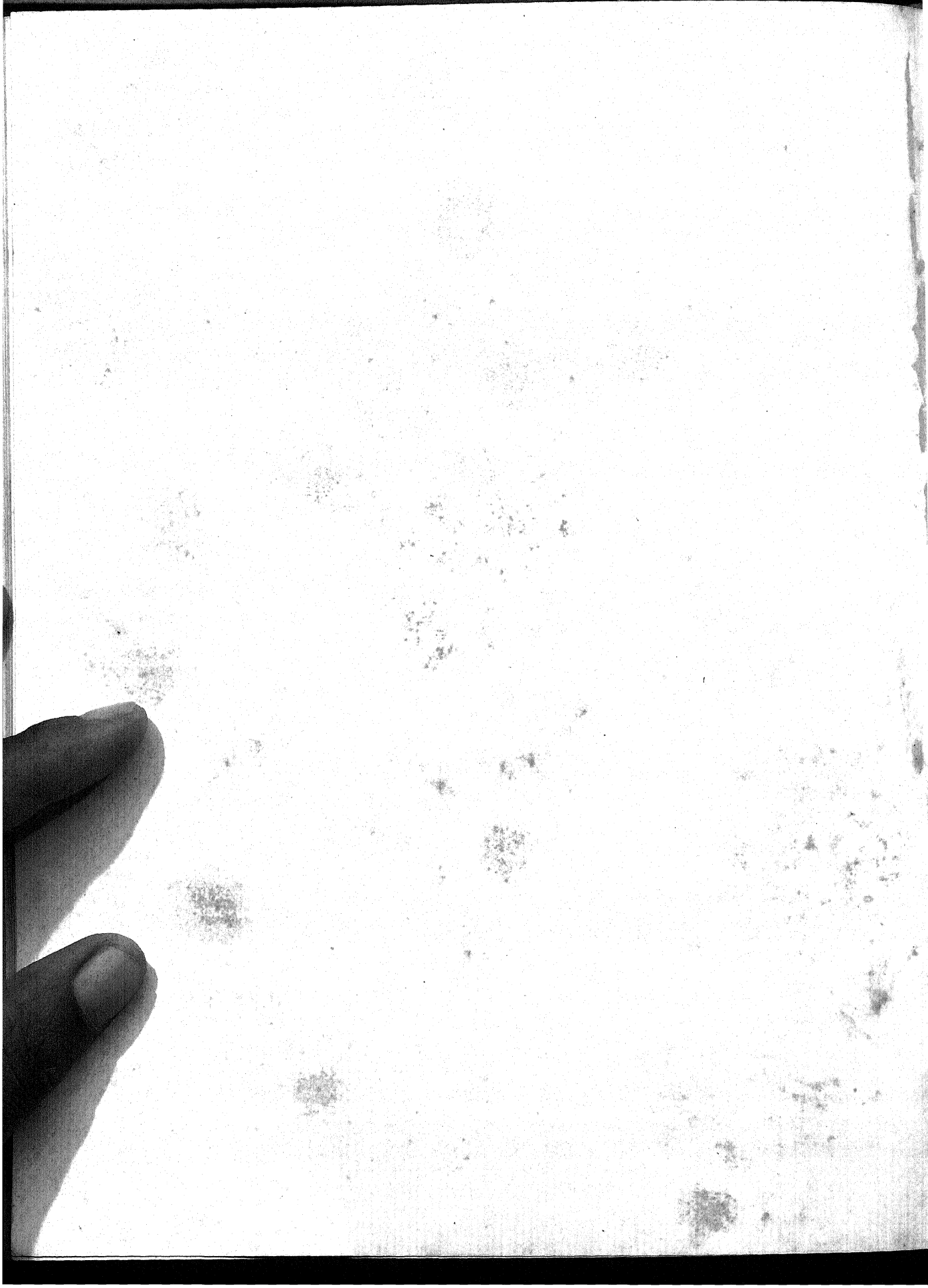
DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to $\frac{1}{4}$. Middle—Reduction to $\frac{1}{2}$. Bottom—Original size.

Reprinted by permission of the authors from: RIKER, A. J., and REGER, S. R. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.



NEW MARINE ALGAE FROM SOUTHERN CALIFORNIA. III¹

George J. Hollenberg

FURTHER STUDY of the marine plants of Southern California (cf. Hollenberg, 1940, 1943) brings to light additional plants which seem not to have been previously reported. Several of these are described herewith.

Griffithsia multiramosa (Setchell and Gardner) Taylor, (*Neomonospora multiramosa* Setchell and Gardner, 1937) var. **balboensis**, var. nov. (fig. 1). Plantae rosaceorubrae aut lucidae rubra, cristas delicatas et globosas et 10–15 cm. crassis formantes, fere epiphyticae, implicatae et dense ramosae; ramis multo dichotomo ramosis; 150–170 μ crassis deorsum et gradatim angustioribus sursum ad 30 μ aut minus; segmentis fere 10–18 diametros longis, et minus ad apicem ramorum, leviter tumidis; tetrasporangiis sparsis, globosis et 60–70 μ crassis, tetrahedralibus divisis, sine ramis involucralibus, singulatim per pediculum fere unicellulare affixis; cystocarpis et ramis antheridialibus ignotis.

Plants rose-red to deep red in color, forming delicate globular tufts mostly 10–15 cm. in diameter, epiphytic or often unattached, intricately and densely branched; branches repeatedly dichotomous at a very narrow angle except in lower parts, 150–170 μ in diameter and with relatively thick walls below and gradually narrower above to 30 μ or less, with thin walls; segments mostly 10–18 diameters long and shorter toward the tips of the branches, only slightly tumid; tetrasporangia infrequent, globular and 60–70 μ in diameter, tetrahedrally divided, without involucral branches, attached singly mostly at non-forking nodes by a short pedicel of one or two cells, so that a tetrasporangial branch forms one branch of a dichotomy; sexual plants unknown; plants occurring in sheltered water of inland bays.

The type specimen of this plant is number 2364, tetrasporic, collected by the writer, September 24, 1938, near the bridge of State Highway No. 101, where the upper bay connects with the harbor proper at Balboa, Orange County, California. It has been repeatedly collected at this place where it is sometimes very abundant and either floating free in the tidal current or attached insecurely to various objects. This plant has been collected by various investigators a number of times at different places in Southern California over a period of many years, but is rarely found in fruit. The scarcity of reproductive structures may bear some relation to the seeming readiness with which the plant propagates vegetatively. Rhizoids arise with considerable frequency from the branches and the writer is inclined to believe that fragmentation followed by ready formation of rhizoids results in vegetative propagation, although no experiments have been performed in this connection.

¹ Received for publication May 10, 1945.

G. multiramosa var. *balboensis* is very close to *G. multiramosa* var. *minor* described by Taylor (1939) from Baja California, Mexico. The local plant is much larger and differs in its more globular form and frequently free-floating habit, and especially in habitat, since it seems to be confined to the warm water of sheltered bays and lagoons. From the species as described by Setchell and Gardner (1937) the plant differs in the tetrahedral division of the sporangia and in habit and habitat. It also seems close to *G. arachnoidea* described by C. Agardh (1828) from the coast of "Gallea" and figured by Børgesen (1930). Judging by Agardh's description, and by the figures and description of Børgesen, the California plant differs in the more globular form and less tumid cells. Plants collected by Børgesen were from exposed positions. Southern California plants seem to be decidedly limited to warm, sheltered bays.

CHONDRIA arcuata sp. nov. (fig. 2–4) Frondes pullo rubrae et cristatae, cum ramis prostratis et repentibus per intervalla frequentia per hapteres breves et robustos ad saxa affixis et cum ramis erectis 3–4 cm. altis et 300–400–(700) μ crassis; ramis perspicue arcuatis, ad fundamentum attenuatis et fere cum ramulis paucis et similiter arcuatis; ramis manifeste polysiphonis, cum quinque cellulis pericentralibus comparate magnis, longitudine aequali; segmentis axis pericentralibus fere 1–1.5 diametros longis; cortice tenui circiter stratorum duorum cellularum 20–25 μ crassis et 1.0–1.5–(4) diametros longarum constructo; apicibus crescentibus in puteis in apicibus ramorum truncatorum positis, et cristam trichoblastarum brevium et dense furcatarum ferentibus; tetrasporangiis in apicibus ramorum sitis, 80–90 μ crassis, tripartitis; cystocarpis ovoidis et sessilibus, circiter 360–450 μ crassis, sine calcaribus in basi; ramulis antheridialibus orbicularibus numerosis ad apices ramorum, 180–215 μ latis.

Fronds dull red, tufted, with prostrate creeping branches attached to rocks by short sturdy hapteres at frequent intervals and erect terete branches 3–4 cm. high and 300–400–(700) μ in diameter; branches conspicuously arcuate, attenuate at the base and mostly with few or no branchlets, the branchlets being similarly curved; branches conspicuously polysiphonous internally, with five relatively large pericentral cells of uniform length showing plainly through the relatively thin cortex and giving an evidently segmented appearance to the branches with respect to the inner core; segments of the pericentral axis composing more than half of the width of the branches and 1.0–1.5 diameters long; cortex mostly of 2–3 layers of cells 20–25 μ in diameter and 1.0–1.5–(4.0) diameters long; chromatophores in the form of long, contorted bands; growing point of

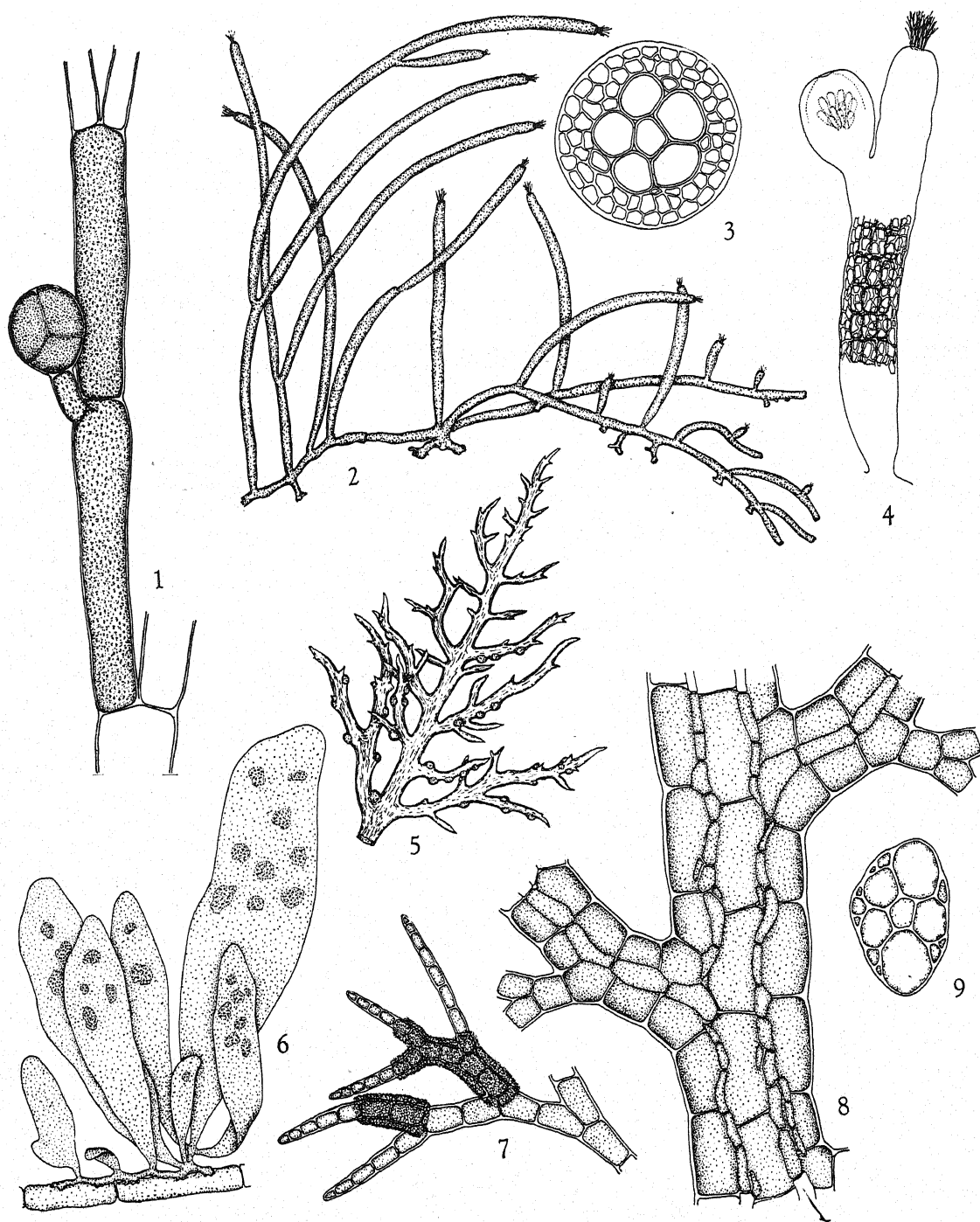


Fig. 1-9. All figures except the habit sketches represented in figures 2, 5, and 6 were drawn with the aid of a camera lucida.—Fig. 1. Portion of a branch of *Griffithsia multiramosa* var. *balboensis* var. nov. showing tetrasporangium. $\times 215$.—Fig. 2. Habit sketch of *Chondria arcuata* sp. nov. $\times 6$.—Fig. 3. Cross section of a branch of *C. arcuata* $\times 100$.—Fig. 4. Branch of *C. arcuata* showing cystocarp and pericentral cells. $\times 25$.—Fig. 5. Habit sketch of a portion of a cystocarpic plant of *Gigartina tepida* sp. nov. $\times 3.5$.—Fig. 6. Habit sketch of a tetrasporic plant of *Myriogramme repens* sp. nov. $\times 10$.—Fig. 7. Antheridial branch of *Heterosiphonia asymmetria* sp. nov. $\times 430$.—Fig. 8. Portion of a branch of *H. asymmetria* showing cortical filaments. $\times 100$.—Fig. 9. Cross section of a branch of *H. asymmetria*. $\times 100$.

branches in a pit at the tips of the truncate branches and bearing a tuft of short, densely-branched trichoblasts; tetrasporangia $80-90\ \mu$ in diameter, embedded in the tips of the branches, tripartitely divided; cystocarps ovoid sessile, about $360-450\ \mu$ in size, without a basal spur; antheridial discs oval, $180-215\ \mu$ wide, abundant at the tips of the branches. Plants attached to rocks along with *Spermothamnion Snyderae* Farlow and other diminutive plants at low tide level, Laguna Beach and Corona del Mar.

The type is number 1058 collected by the writer at Laguna Beach, Orange County, California, on October 29, 1935. This relatively small member of the genus has been found a number of times in the above mentioned localities. It seems to be the second species in the subgenus *Coelochondria* so far reported from the Pacific Coast of North America. The curving branches, the stoloniferous habit and the thinly corticate pericentral cells of uniform length seem to be the most distinctive features. In certain of these respects as well as in size and general habit the present species resembles *C. curvilineata* described by Collins and Hervey (1917) from Bermuda. However, the latter plant is characterized by prominently thickened and curving walls at the ends of the pericentral cells. *C. arcuata* also resembles *C. simplisuscula*, described by Madame Webber-van Bosse (1913), but the latter plant is epiphytic and is not described as having curving branches.

GIGARTINA *tepida* sp. nov. (fig. 5) Plantae laxae cristatae, ad 9 cm. altae, 3-4 plo alterne ramosae in uno plano in modo decomposito cum axibus principibus complanatis, $0.5-1.0\ \text{mm.}$ latis et plus aut minus distinctis et flexis; ramis ultimis distichis, acutis et fere 5 mm. longis; soris tetrasporangiorum parvis et superficialis, fere ad margine ramulorum; cystocarpis ad $500\ \mu$ crassis fere ad margine ramulorum; soris antheridialibus parvis.

Plants loosely tufted, to 9 cm. high, 3-4 times alternately branched in one plane in a decompositely pinnate manner, with more or less distinct but flexuous leading axes; main branches $0.5-1.0\ \text{mm.}$ in diameter, complanate, with distichous and sharply pointed ultimate branches, mostly about 5 mm. long; tetrasporangial sori small and superficial, mostly marginal on the ultimate and subultimate branches; cystocarps to $500\ \mu$ in diameter, mostly marginal on branches of all orders; plants attached to shells, stones, and woodwork in sheltered bays.

The type specimen, number 1157 in the herbarium of the writer, is tetrasporic and was collected among others from shells and rocks near the bridge by which the state highway number 101 crosses the outlet to the upper harbor at Balboa in Orange County, California, on December 8, 1935. This plant has been collected repeatedly at this locality, which is subject to tidal currents, but does not seem to occur along the open coast in this region. Specimens collected by Mr. E. Yale Dawson (1944) from the Gulf of California, and identified as *G. Teedii* (Roth) Lamour., are possibly identical with the writer's specimens. It seems likely that this plant is

limited to warm sheltered water more or less agitated by tidal currents.

This species is the only local representative of the *Aciculares* section of the genus as outlined by Setchell and Gardner (1933) in accordance with the original divisions made by Agardh (1899). Judging by the few specimens of *G. Teedii* available for examination, the California plant appears to differ from that plant in habitat, in the branching habit with less distinct main axes, and in the more distinctly slender medullary filaments. Furthermore, spinose branchlets seem to arise frequently or at least occasionally from the flat faces of the branches in the case of *G. Teedii*, but such branches seem never to occur in the local plant.

MYRIOGRAMME *repens* sp. nov. (fig. 6) Frondes erectae e fundamento cylindrato et repente cum hapteris pluribus; divisionibus erectis fere $5-8\ \text{mm.}$ altis et $1.5-2.0\ \text{mm.}$ latis, simplicibus aut cristatis cum pluribus ramis ad fundamentum, monostromaticis extra in areas fructiferas, in parte media $60-88\ \mu$ crassis, ex cellulis $30-50\ \mu$ crassis compositis; tetrasporangiis $50-65\ \mu$ crassis, in soris dispersis $400-500\ \mu$ crassis et plus aut minus orbicularibus; cystocarpis paucis, dispersis, $500-600\ \mu$ crassis cum ostiolo ad uno plano; soris spermatangialibus paucis et dispersis aut areas irregulares ad apices frondum formantibus.

Fronds erect from a cylindrical creeping and hapterous base; erect blades mostly $5-8\ \text{mm.}$ high and $1.5-2.0\ \text{mm.}$ wide, simply or slightly proliferous—tufted from the base, monostromatic, except in fruiting areas where it is composed of 3 layers of cells, $60-80\ \mu$ thick in the center and thinner toward the margins, composed of cells $30-50\ \mu$ in diameter; tetrasporangia $50-65\ \mu$ in diameter, in more or less circular scattered sori $400-500\ \mu$ in diameter; cystocarps few, scattered, $500-600\ \mu$ in diameter, with a short conical bulge terminated by a pore on one side of the thallus and with a low rounded bulge on the other side; spermatangial sori few and scattered or forming a single irregular patch toward the tip of a blade.

The type material (Hollenberg number 2424) was collected from corallines along with *Cryptopleura corallinara* (Nott) Gardner at low tide level near Pt. Vicente, south of Redondo Beach, Los Angeles County, California, December 6, 1938. The plant was again collected in this vicinity a year later. This diminutive species is similar to *M. Hollenbergii* Kylin (1941), which the writer collected at Monterey. The southern plant is distinguished by its smaller size and its general shape and branching, but especially by the creeping rhizome.

HETEROSIPHONIA *asymmetria* sp. nov. (fig. 7-9) Frondes erectae a ramis prostratis, vel $2\ \text{cm.}$ altae, cum axibus principibus compressis, $200-290\ \mu$ latis, et cum segmentis latitudine haud longioribus; cellulis pericentralibus quinque, non transverse divis, cum pericentralibus cellulis lateralibus maioribus et constanter cum una pericentrali cellula minore in altero latere ramorum complanatorum et duabus in

altero latere; ramis invicem distichis, cum 2-3 segmentis inter ramos succedentes; ramulis 1-2 mm. longis, ultimos ramulos monosiphonos et attenuatos cum apicibus rotundis et e (6)-9-12 cellulis 1-2 diametros longis ferentibus; axibus principibus leviter corticatis per filamenta longitudinalia lineas juncturarum cellularum pericentralium tegentia; antheridiis in omnibus lateribus plurium cellularum ad fundamenta ramulorum ultimorum et subultimorum cum apicibus sterilibus; tetrasporangiis et cystocarpis ignotis; plantae ad saxa affixae.

Fronds erect from prostrate branches, to 2 cm. high; main axes compressed, 200-290 μ wide, of segments scarcely longer than wide; pericentral cells five, never transversely divided, of unequal size, with two larger ones marginal in position and three smaller pericentral cells. Two of the latter are constantly one on one face and one on the other face of the flattened branches; all branching alternate in one plane, with 2-3 segments between successive branches; branchlets 1-2 mm. long bearing tapering monosiphonous ultimate branchlets with rounded tips and composed of (6)-9-12 cells 1-2 diameters long; main axes slightly corticated by longitudinal filaments covering the line of juncture of the pericentral cells; antheridia covering continuously all sides of a number of cells at the bases of sterile-tipped ultimate and subultimate branchlets; tetrasporangia and cystocarps unknown. Plants attached to rocks at low tide level.

The type (Hollenberg 2253) is the only known material. It was collected at low tide level on rocks near Corona del Mar, Orange County, California, February 12, 1938. The five asymmetrically arranged pericentral cells seem distinctive. In general aspect the plant resembles *H. erecta* described by Gardner (1927), and common along the coast of southern California. However, it is readily distinguished from that species by the number and arrangement of the pericentral cells, by the compressed axes, and by the cortical filaments. It is also much more flaccid than *H. erecta*, with more slender monosiphonous ramuli. In the flattened segments with larger marginal pericentral cells *H. asymmetria* resembles the type species *H. Berkleyi* described by Montagne (1842), but the latter is usually without cortication and has symmetrically arranged pericentral cells, often 8 in number.

PORPHYRELLA californica sp. nov. Plantae pallidae vel medae fusco-rubrae, iridescentae ovatae vel orbiculatae ad fundamentum cordatae aut, interdum elongatiores cum fundamento cuneato, praecipue si juvenes, per stipite breve affixae qui ex filamentis rhizoidis e cellulis inferioribus compositus est; thallus ad 25 mm. latis, monostromaticis, 15-20 μ crassis, cum cellulis dense aggregatis et 5-10 μ crassis; spermatangiis 32-64 in quoque fasciculo in soris parvis irregularibus et dispersis ad marginem thalli; plantae fere non epiphyticae.

Plants medium to pale reddish brown iridescent, oval to orbicular and commonly cordate at the base or frequently more elongate with cuneate base when

young, attached by a very short rhizoidal stipe; thalli up to 25 mm. broad, monostromatic, 15-20 μ thick and composed of closely crowded cells 5-10 μ in diameter and slightly longer in vertical section; cells each with a stellate chromatophore; spermatangia in packets of 32-64 in small irregular scattered sori near the margin of the thallus; carpogonia marginal, forming between spermatangial areas, liberated as undivided cells (carpospores?); plants attached to rocks, limpets, colonial hydrozoans and only infrequently to other algae.

The type material (Hollenberg number 1353) was collected from rocks, limpets, and colonial hydrozoans from the midlittoral zone near Lady's Harbor on the north side of Santa Cruz Island off the coast of Southern California, April 19, 1936. The plant has also been collected at several places along the mainland coast of Southern California.

Porphyrella Gardneri, described by Smith and Hollenberg (1943) occurs along the coast of central California and as far north as the coast of Oregon. It differs from the present species chiefly in its epiphytic habit and shape of thallus, which is more constantly cuneate.

The northern plant seems never to have the cordate base so frequently characteristic of *P. californica*.

PETROGLOSSUM parvum sp. nov. Plantae fere 10-18 mm. altae, simplices aut fere cum nonnullis ramis lateralibus et simplicibus ad fundamenta; ramis ligulaformibus 0.8-1.5-(2.0) mm. latis et 60-120 μ crassis; soris tetrasporangialibus et antheridialibus circularis et parvis, fere singulis et fere in centrum ramorum positiss; cystocarpis fere in centrum ramorum positiss.

Plants 10-18 mm. high, simple or mostly with a few simple lateral branches from near the base; branches ligulate, 0.8-1.5-(2.0) mm. wide and 60-120 μ thick; tetrasporangial and antheridial sori small and circular, about half the width of the branches, occurring mostly singly near the center of the branches, a new tetrasporangial sorus frequently appearing in terminal proliferations before an old one has completely disappeared in the main blade; cystocarps similarly located and likewise occurring singly; plants frequent on rocks at low tide level along the coast of southern California.

The type material (Hollenberg number 672, tetrasporic) was collected at low tide level at Laguna Beach, Orange County, California, March 1, 1935.

In a recent communication, Lt. E. Yale Dawson reports finding a plant in the vicinity of La Jolla, which he concludes is *P. parvum* after examining some of the type collection. He also reports finding cystocarpic plants of the larger species, *P. pacificum*, previously described by the writer (1943). His collections confirm the writer's conviction, based on many collections over a period of 10 years, that the smaller plant should be considered a new species as suggested in the original description of *P. pacificum*, although the male plant figured in that description (fig. 1), is confusingly intermediate in size and

branching. Tetrasporic plants of *P. pacificum* are still unknown. However, that species is not only larger and more abundantly branched, with thicker branches, but the cystocarps and antheridial sori usually occur near the branch tips or on small pro-

liferous branches in *P. pacificum*, rather than occupying a more central position as in *P. parvum*.

DEPARTMENT OF BIOLOGY,
UNIVERSITY OF REDLANDS,
REDLANDS, CALIFORNIA

LITERATURE CITED

- AGARDH, C. 1828. *Species algarum*. Stockholm.
- AGARDH, J. G. 1899. *Analecta Algologica: observationes de speciebus minus cognitis earumque dispositionis*. IV. Actis Soc. Physiog. Lundensis, N.S. 8: 1-106.
- BØRGENSEN, F. 1930. Marine algae from the Canary Islands. Part III, Rhodophyceae. Biol. Meddel. Kgl. Dansk Vidensk. Selsk. 19: 1-158.
- COLLINS, F. S., AND A. B. HERVEY. 1917. The algae of Bermuda. Proc. Amer. Acad. Arts and Sci. 53: 3-195.
- DAWSON, E. Y. 1944. The marine algae of the Gulf of California. Allan Hancock Pacific Expeditions 3(10): 189-464.
- GARDNER, N. L. 1927. New Rhodophyceae from the Pacific Coast of North America VI. Univ. Calif. Publ. Bot. 14: 99-138.
- HOLLENBERG, G. J. 1940. New marine algae from Southern California. I. Amer. Jour. Bot. 27: 868-877.
- . 1943. New marine algae from Southern California. II. Amer. Jour. Bot. 30: 571-579.
- KYLIN, H. 1941. Californische Rhodophyceen. Lunds Univ. Arsskr. N.F., Avd. 2, 37: 1-51.
- MONTAGNE, C. 1842. *Prodromus generum specierumque Phycarum novarum, in itinere ad polum antarcticum*. Paris.
- SETCHELL, W. A., AND N. L. GARDNER. 1933. A preliminary survey of *Gigartina*, with special reference to its Pacific North American species. Univ. of Calif. Publ. Bot. 17: 255-340.
- . 1937. The Templeton Crocker expedition of the California Academy of Sciences, 1932. No. 31. A preliminary report on the algae. Proc. Calif. Acad. Sci. 22(2): 65-98.
- SMITH, G. M., AND G. J. HOLLENBERG. 1943. On some Rhodophyceae from the Monterey Peninsula, California. Amer. Jour. Bot. 30: 211-222.
- TAYLOR, W. R. 1939. Algae collected on the presidential cruise of 1938. Smithsonian Misc. Collections 98(9): 1-18.
- WEBBER-VAN BOSSE, A. 1913. Marine algae of the Sealark expedition. Trans. Linn. Soc. London, Botany, II 8: 105-142.

CYTOGENETICS OF CERTAIN TRITICUM-AGROPYRON HYBRIDS AND THEIR FERTILE DERIVATIVES¹

R. Merton Love and C. A. Suneson

IN 1938, Mr. W. J. Sando, U. S. Department of Agriculture, sent certain Triticum-Agropyron material to Davis, California, for observation and study. Included were two hybrid seeds of *Triticum durum* var. Mindum \times *Agropyron trichophorum* and two of *T. macha* \times *A. trichophorum*. The cytogenetics of these hybrids and some fertile derivatives therefrom will be discussed in this paper.

MATERIAL.—*The parents.*—Unfortunately, there is little information about the parents and what there is, is rather indirect. *T. macha* Dekapr. and Menabde ($2n = 42$) was found in Georgia, U.S.S.R. (Dekaprevitch and Menabde, 1932). It was considered by them to be one of the progenitors of *T. spelta* L., but Chin and Chwang (1944) pointed out that while *T. macha* resembles *T. spelta* in the toughness of its glumes, it differs from the latter in that the rachis fractures above the point of attachment of the spikelets and it has short awns. They reported finding up to 21 bivalents in hybrids between these two species, but meiosis was irregular. *A. trichophorum* (Link) Richt. has been reported as having 42 chromosomes (Araratian, 1938) but its chromosomes have not been studied during meiosis. It has been mentioned in various reports on hybridization projects between wheats and Agropyrons (White, 1940; Smith, 1942;

Zhebrak, 1944), but apparently it has been one of the less successful parents in such crosses.

The first generation plants.—Two F_1 plants of the cross *T. durum* var. Mindum ($2n = 28$) \times *A. trichophorum* ($2n = 42$) were established. They were quite similar morphologically and in their rust reactions (table 1). Each had the expected number of 35 chromosomes, but the behavior of the chromosomes during meiosis indicates a marked cytological difference between the two plants (table 3).

Two F_1 plants of the cross *T. macha* ($2n = 42$) \times *A. trichophorum* ($2n = 42$) were established. These were similar morphologically and in their rust reactions (table 1). The one that set six seeds in 1940 died in 1943. This plant was not examined cytologically, but presumably it had 42 chromosomes. The other plant, which had set no seed, had only 41 chromosomes (table 3).

Fertility of all hybrids was extremely low; only a few seeds were set despite the annual production of 300 or more spikes by each plant. Beginning in 1942, even more spikes have been produced annually, for each plant was vegetatively divided into 11 clones. This transplanting changed associations and proximities to other hybrids and may have influenced fertility. Natural crossing with adjacent plants is possible since the sterile florets remain open, and the stigmas receptive, for from six to ten days. On the

¹ Received for publication May 21, 1945.

TABLE 1. *The first generation plants.*

Cross and accession number	2n	Number seeds set						Rust reaction	Spike characters		Number of plants obtained
		'39	'40	'41	'42	'43	'44		Rachis Artic.	Glumes	
<i>Triticum durum</i> var. Mindum											
× <i>Agropyron trichophorum</i>											
37306-1	35	1	12	0	0	0	0	very resistant	fragile above	Pub.	6 (198c)
37306-2	35	0	20	2	0	0	0	very resistant	fragile above	Pub.	5 (198d)
<i>Triticum macha</i> ×											
<i>A. trichophorum</i>											
37308-1	42?	0	6	0	0	0	..	very resistant	fragile above	Pub.	1 (198e)
37308-2	41	0	0	0	0	0	0	very resistant	fragile above	Pub.	0

individual plants, flowering proceeded over a period of a month or more in each season. It should be pointed out that if natural crossing occurred, it was likely confined to these and other F_1 plants.

The fertile derivatives.—From 13 and 22 seeds set by the two *T. durum* var. Mindum × *A. trichophorum* hybrids, only six and five plants, respectively, were established in each line. With two exceptions, they were about as vigorous as their respective hybrid parents and morphologically similar to them, although stems, leaves, and spikes were individually larger on the fertile derivatives. They were somewhat less resistant to leaf and stem rusts (table 2). Their seeds were light red, blue, or grey. They ranged in sizes from nearly as small as the grass parent to the equal of Mindum. Relatively few were of normal plumpness.

From the six seeds set on the *T. macha* × *A. trichophorum* hybrid, only 1 plant was established. This plant resembled the original hybrid in its very high resistance to both rusts, but it differed in having glabrous glumes, a semi-fragile rachis, and blue or red seeds intermediate between the parents in size and plumpness (table 2). It had 70 chromosomes (table 3).

Although all the derivatives, except 198c-1 and 198c-6, set many more seeds than did the original

hybrids, there is a marked variation in fertility of the different plants. Thus, the annual seed production of 14 grams for plant 198d-2 is expressed as a relative fertility index of 150 (table 2). Non-viable seeds are commonly produced by these plants.

CYTOLOGICAL ANALYSIS.—Of the 12 plants in table 2, five have been examined cytologically and compared with the F_1 plants (table 3). Material was collected and slides prepared as previously reported (Love, 1940). Meiotic material of the three F_1 plants was collected the same afternoon so that any cytological differences observed must be intrinsic. Material of the other plants was collected at different times so that comparisons here are not so accurate. Fifty nuclei at first metaphase were completely analysed and 200 tetrads were examined in each plant. The photomicrographs were taken at a magnification of ×580.

T. durum var. Mindum ($2n = 28$) × *A. trichophorum* ($2n = 42$).—As seen by the data summarized in table 3, the two F_1 plants differed markedly in their meiotic chromosome behavior (fig. 1 and 2). Whereas the first had an average of 1.60 pairs with a maximum of four pairs, the second had an average of 6.06 with a maximum of 10 pairs and a chain of three chromosomes. In the former plant the 35 chromosomes were paired in five different ways in the

TABLE 2. *The fertile derivatives.*

F ₁ parent	Progenies established	Longevity (years)	Spike characters		Seed characters		Rust reaction	Relative fertility
			Rachis	Glumes	Color	Size index		
37306-1 ^a	198c-1	1	This plant was a sterile dwarf					0
	-2 ^a	3	Fragile	Pubescent	Light red	4-10	Susc. to leaf	75
	-3	4+ ^b	Fragile	Pubescent	Light red	2-9	rust; moder-	15
	-4	3+	Fragile	Pubescent	Red or grey	3-10	ate resist. to	30
	-5	3+	Fragile	Pubescent	Red or grey	2-10	stem rust	25
	-6 ^a	1	?	?	?	?		0
37306-2 ^a	198d-1	4+	Fragile	Pubescent	Blue or grey	2-6	Res. to leaf	37
	-2 ^a	4+	Fragile	Pubescent	Red, blue, or grey	5-8	rust	150
	-3 ^a	4+	Fragile	Pubescent	Red or blue	3-6	moderate	25
	-4	4+	Fragile	Pubescent	Light red	4-8	res. to	50
	-5	3+	Fragile	Pubescent	Light red	4-8	stem rust	37
37308-1	198e-1 ^a	4+	Semi-fragile	Glabrous	Blue or red	5	Res. to both rusts	100

^a Plants studied cytologically.

^b Plus sign means the plant was alive January 13, 1943, and subsequently plowed up (3+) or transplanted (4+).

TABLE 3. Summary of meiotic chromosome behavior.

	<i>T. durum</i> var. <i>Mindum</i> × <i>A. trichophorum</i>		<i>T. macha</i> × <i>A. trichophorum</i>	Fertile derivatives ^a				
	37306-1	37306-2	37308-2	ex 37306-1 198c-2	198c-6 ^a	198d-2	198d-3	ex 37308-1 198e-1
2n	35	35	41	56	35	70	70	70
Univalents								
Range	27-35	12-27	13-31	15-26	21-35	9-30	19-41	14-33
Average	31.80	20.22	22.64	21.10	28.16	19.56	28.34	22.64
Bivalents								
Range	0-4	3-10	4-13	14-20	0-6	17-27	11-22	14-24
Average	1.60	6.06	7.70	16.58	3.26	21.88	17.72	18.62
Closed bivalents								
Range	0-1	0-2	0-3	3-11	0-1	4-17	2-12	3-10
Average	0.02	0.32	1.10	7.80	0.16	10.78	6.94	7.03
Chains of three								
Range	0-3	0-3	0-2	0-2	0-5	0-4	0-6
Average	0.86	0.88	0.58	0.08	1.80	1.36	2.10
Chains of four								
Range	0-2	0-1	..	0-1	0-2	0-2	0-3
Average	0.32	0.80	..	0.02	0.32	0.48	0.82
Chains of five								
Range	0-1	0-2
Average	0.02	0.06
Chains of six								
Range	0-1	0-1
Average	0.02	0.04
Chromosomes synapsed								
Range	0-8	8-23	13-28	30-41	0-12	40-61	29-51	37-56
Average	3.20	14.78	18.36	34.90	6.84	50.44	41.66	47.36
Chromosomes involved in multiple associations								
Range	0-9	0-10	0-6	0-6	0-17	0-14	3-20
Average	2.66	5.84	4.74	0.32	6.68	6.22	10.12
Number of different pairing arrangements ^b	5	20	27	14	11	30	36	44
Percentage irregular pollen tetrads	100	93	90	100	?	100	89	100
Micronuclei per tetrad								
Range	1-7	0-7	0-6	1-12	?	2-18	0-16	1-15
Average	3.35	2.31	2.11	5.90	?	9.08	2.59	5.96
Percentage cells with one or more inversion bridges	0	4	25	0	?	1	49	9

^a Plant 198c-6 was sterile.^b See explanation in the text.

50 cells examined. In the latter there were 20 different pairing arrangements. Closed bivalents were rare in both plants.

Little is known of the chromosomal constitution of the parents. Thus, it is impossible to decide whether the differences found in the two F_1 plants should be ascribed to strain differences in the parents used in this particular cross, or to genes which reduce pairing in one of the plants. At any rate, the parents have few chromosomes in common, and the lack of ring bivalents in the hybrids indicates that what pairing occurs is due to homology of parts of chromosomes (homoeology) rather than of whole chromosomes.

The fertile derivatives.—Meiotic chromosome behavior in these plants is very complex (table 3). Two of the progeny of 37306-1 were examined cyto-

logically. One of these, 198c-6, had 35 chromosomes (fig. 3) and was sterile. Pairing in this plant, though weak, was twice that in its parent. This plant is mentioned because it indicates one of two possibilities: either an unreduced, unfertilized gamete was functional, or two partially reduced gametes combined to produce a fairly viable plant.

The other plant, 198c-2, had 56 chromosomes (fig. 4) with a maximum of 20 pairs of which eleven were ring bivalents. In this instance, it is obvious that at least one partially reduced gamete was involved. The minimum number of univalents in 198c-2 was 15.

The two fertile derivatives, 198d-2 and 198d-3, progeny of 37306-2, had the amphidiploid number of chromosomes ($2n = 70$), but the meiotic chromosome behavior does not resemble that expected

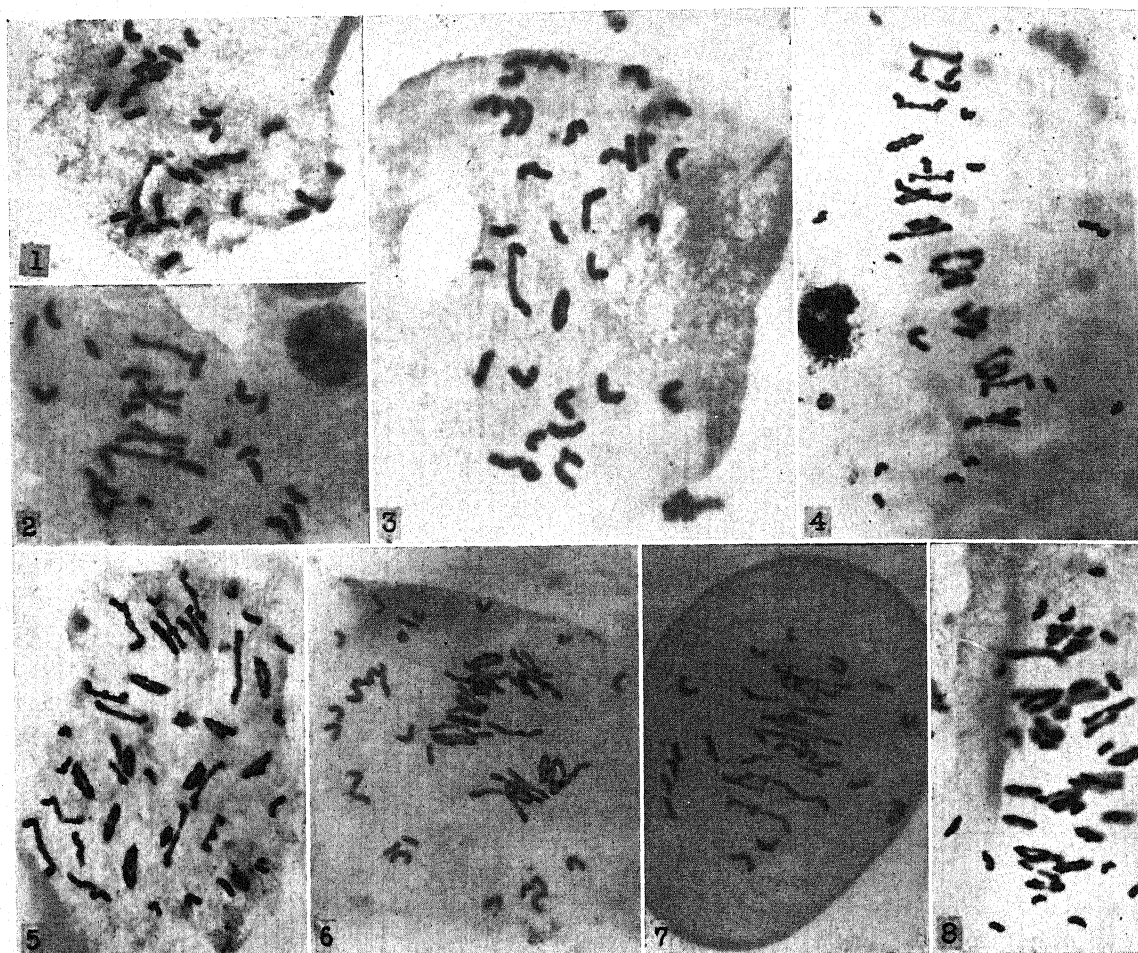


Fig. 1-8.—Aceto-carmine smears of first meiotic metaphases. $\times 580$.—Fig. 1-2. *T. durum* var. Mindum \times *A. trichophorum* F_1 ($2n = 35$).—Fig. 1. Plant 37306-1. $1_{II} + 33_I$.—Fig. 2. Plant 37306-2. $6_{II} + 23_I$.—Fig. 3-6. Derivatives of Mindum \times *A. trichophorum*.—Fig. 3. Plant 198c-6 ($2n = 35$). $3_{II} + 29_I$ (1 closed pair).—Fig. 4. Plant 198c-2 ($2n = 56$). $18_{II} + 20_I$ (8 closed pairs).—Fig. 5. Plant 198d-2 ($2n = 70$). $2_{III} + 24_{II} + 16_I$ (9 closed pairs).—Fig. 6. Plant 198d-3 ($2n = 70$). $2_{III} + 18_{II} + 28_I$ (10 closed pairs). Note the ring univalent.—Fig. 7. *T. macha* \times *A. trichophorum* F_1 Plant 37308-2 ($2n = 41$). $12_{II} + 17_I$ (1 closed pair).—Fig. 8. Derivative of *T. macha* \times *A. trichophorum*. Plant 198e-1 ($2n = 70$). $3_{III} + 21_{II} + 19_I$ (15 closed pairs).

in amphidiploids. In an artificially induced amphidiploid of *Aegilops cylindrica* Host ($2n = 28$) \times *Triticum durum* ($2n = 28$), Sears (1944) found an average of 3.6 univalents with a range of from 0 to 12, which he considered abnormally high. He did, however, find some nuclei with the expected 28 pairs. In neither 198d-2 (fig. 5) nor 198d-3 (fig. 6) were 35 bivalents seen. Rather, 27 was the greatest number observed in the former, and 22 in the latter plant. Furthermore, the maximum number of closed pairs (members of which might be considered to be more or less completely homologous) was 17 in 198d-2 and only 12 in 198d-3 (table 3). The former plant had a minimum of 9, and the latter a minimum of 19 univalents. It is difficult to attribute the $2n$ number of these plants to somatic doubling in the hybrid. It seems more reasonable to suppose that 198d-2 resulted from the union of unreduced gam-

etes, the unreduced male gamete being contributed by 37306-1. This assumption is corroborated by the high fertility of 198d-2 (table 2) and the pairing arrangements observed.

The "sister" plant, 198d-3, had a significantly smaller number of synapsed chromosomes and it was only one-sixth as fruitful as 198d-2. In it was seen by far the greatest frequency of inversion bridges (table 3). It was characterized by a "ring" univalent (fig. 6). It is assumed that the unreduced egg in this instance was fertilized by a partially reduced male gamete from another hybrid, perhaps 37308-1. If this did occur, most of the chromosomes in the male gamete must have been *Agropyron* rather than *Triticum* chromosomes. Plant 198d-3 was the smallest, as well as the least fertile of the 198d derivatives. It was intermediate, morphologically, between 198d-2 and 37306-2.

T. macha ($2n = 42$) \times *A. trichophorum* ($2n = 42$).—The cytological picture is somewhat clouded by the fact that the F_1 hybrid examined (37308-2) had only 41 chromosomes (fig. 7). A maximum of three closed pairs observed indicates that the parents have very few chromosomes in common, even though there was sufficient homology to give a maximum of 13 pairs (table 3). No seed has been set by this plant.

The fertile derivative.—Plant 198e-1 was derived from a sister plant, 37308-1 (which presumably had 42 chromosomes), and had 70 chromosomes (fig. 8) with a maximum of 24 pairs, only 10 of which were ring bivalents. As many as 20 chromosomes were involved in multiple associations in some nuclei. As many as six chains of three were observed in some cells, and occasionally three chains of four chromosomes occurred. A total of 44 different pairing arrangements were observed in the 50 nuclei examined (table 3). The plant was fairly fertile and as rust resistant as its female parent. The pairing behavior of the chromosome suggests that 198e-1 may have resulted from the fusion of an unreduced egg and a partially reduced (28-chromosome) male gamete from an F_1 hybrid involving *T. durum* Desf. or *T. aestivum* L. This would also account for the semi-fragile rachis possessed by 198e-1.

Discussion.—It would seem obvious, from the array of data presented above, together with the theoretical considerations arising therefrom, that it would be futile to attempt to ascertain the chromosomal constitution of the fertile derivatives on the basis of pairing by chromosome sets. The results reported above emphasize the statements of Thompson (1931), Dobzhansky (1941), and Love (1941), that extreme caution must be used. However, it must be admitted that such analyses have supplied in the past (see review by Thompson, 1931), and still are supplying (Sears, 1944; Stebbins, Tobgy, and Harlan, 1944), definite clues concerning genetic and taxonomic relationships of polyploid species.

The sterile hybrids have been growing during a 6-year period and are vegetatively vigorous, producing more than 300 spikes annually. Yet 38 of the 41 seeds set were obtained in 1940 (table 1). Twelve progeny plants have been established and five have been examined cytologically. Only two (198d-2 and 198d-3) have the expected amphidiploid chromosome number, but the pairing behavior of their chromosomes is not that expected in amphidiploids (table 3; fig. 5 and 6). The remaining three derivatives examined (198c-2, 198c-6, and 198e-1) have not the amphidiploid number of chromosomes (table 3; fig. 3, 4, and 8), and these must have arisen through the fortuitous union of non-reduced or partially reduced gametes.

Thompson and Armstrong (1932) reported that many partially reduced gametes, with only parts of sets of seven chromosomes included, failed to function or at least to produce viable offspring. They concluded that two or three chromosomes of a set disturbed the genic balance in such gametes so that

they were handicapped in competition with gametes containing complete sets of chromosomes. It seems evident from the data presented in this paper that partially reduced gametes with complete sets of seven chromosomes were produced in this material. Although there must have been a very limited production of such gametes, they would have distinct advantages in competition with other gametes.

The production of fertile derivatives by the union of unreduced or partially reduced gametes indicates that such a method of polyploid production is possible (if not common) in nature. This method of polyploid production with its concomitant fertility might be expected to lead to entirely different results from those obtained from somatic doubling in the hybrid followed by normal gamete production. Where polyploid formation depends upon haphazard union of unreduced or partially reduced gametes (the only apparent limitation being that elimination of chromosomes by sets is necessary), stability is not to be expected in the next generation. The cytology of later generations will be published separately, but it may be mentioned here that the 12 plants of the succeeding generation so far examined varied in chromosome number from 60 to 74, with 10 of the plants having less than 70 chromosomes. Armstrong and McLennan (1944) obtained similar results on their artificially induced amphidiploids in other *Triticum-Agropyron* material, so that other techniques may have to be devised for comparing fertile derivatives produced by the two methods.

The study herein reported is not a controlled experiment. A program now under way on the same material includes artificial backcrosses, outcrosses, and doubling of the original hybrids. The results should provide a comparison between the artificial and natural production of fertile derivatives from sterile hybrids.

SUMMARY

There is very little homology between chromosomes of *Triticum durum* var. Mindum ($2n = 28$) and *Agropyron trichophorum* ($2n = 42$). One sterile derivative ($2n = 35$) and three fertile derivatives (with $2n$ numbers of 56, 70, and 70) were examined. The 56-chromosome plant is obviously not an amphidiploid, and the large number of unpaired chromosomes in the other two fertile plants indicates that they, too, are not amphidiploids. Rather, they owe their origin to random fertilization of partially reduced and non-reduced gametes.

There is also little homology between chromosomes of *T. macha* ($2n = 42$) and *A. trichophorum* ($2n = 42$). The one fertile derivative obtained from this hybrid had $2n = 70$, again obviously not an amphidiploid number.

Although the sterile hybrids have been growing during a 6-year period, one seed was obtained in 1939, 38 seeds in 1940, and two in 1941. From the 41 seeds, only 12 plants have been established. Five of these have been examined cytologically and only two have the amphidiploid number of chromosome

(but these do not have the expected amphidiploid chromosome behavior). Allopolyploids, under favorable conditions such as must have obtained in 1940 for this material, can be produced by the fortuitous union of compatible gametes. It is suggested that this method of allopolyploid production may be of more frequent occurrence than is commonly recognized.

DIVISION OF AGRONOMY,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA, AND
DIVISION OF CEREAL CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY, SOILS, AND
AGRICULTURAL ENGINEERING,
AGRIC. RESEARCH ADMINISTRATION,
U. S. DEPARTMENT OF AGRICULTURE,
DAVIS, CALIFORNIA

LITERATURE CITED

- ARARATIAN, A. G. 1938. The chromosome numbers of certain species and forms of *Agropyron*. Societ Bot. 1936:109-111. Seen in Plant Breed. Abst. 9:1080. 1939.
- ARMSTRONG, J. M., AND H. A. McLENNAN. 1944. Amphidiploidy in *Triticum-Agropyron* hybrids. Sci. Agric. 24:285-298.
- CHIN, T. C., AND C. S. CHWANG. 1944. Cytogenetic studies with "mahka" wheat. Bull. Torrey Bot. Club 71:356-366.
- DEKAPRELEVITCH, L. A., AND V. L. MENABDE. 1932. Spelt wheats of western Georgia (western Transcaucasia). Bull. Appl. Bot. Leningrad 1:3-46. Seen in Plant Breed. Abstracts 3:190. 1932-3.
- DOBZHANSKY, T. 1941. Genetics and the origin of species. 2nd Ed. Rev. Columbia University Press, New York. 466 pp.
- LOVE, R. M. 1940. Chromosome number and behaviour in a plant breeder's sample of pentaploid wheat hybrid derivatives. Canadian Jour. Res. C, 18:415-434.
- . 1941. Chromosome behaviour in F_1 wheat hybrids. I. Pentaploids. Canadian Jour. Res. C, 19:351-369.
- SEARS, E. R. 1944. The amphidiploids *Aegilops cylindrica* \times *Triticum durum* and *A. ventricosa* \times *T. durum* and their hybrids with *T. aestivum*. Jour. Agric. Res. 68:135-144.
- SMITH, D. C. 1942. Intergeneric hybridization of cereals and other grasses. Jour. Agric. Res. 64:33-47.
- STEBBINS, G. L., JR., H. A. TOBGY, AND J. R. HARLAN. 1944. The cytogenetics of hybrids in *Bromus*. II. *Bromus carinatus* \times *Bromus arizonicus*. Proc. California Acad. Sci. 4th Series 25:307-322.
- THOMPSON, W. P. 1931. Chromosome homologies in wheat, rye, and *Aegilops*. Canadian Jour. Res. 4:624-634.
- , AND J. M. ARMSTRONG. 1932. Studies on the failure of hybrid germ cells to function in wheat species crosses. Canadian Jour. Res. 6:362-373.
- WHITE, W. J. 1940. Intergeneric crosses between *Triticum* and *Agropyron*. Sci. Agr. 21:198-232.
- ZHEBRACK, A. 1944. Synthesis of new species of wheats. Nature 153:549-551.

ORIGIN AND DEVELOPMENT OF SCLEREIDS IN THE FOLIAGE LEAF OF *TROCHODENDRON ARALIOIDES* SIEB. & ZUCC.¹

Adriance S. Foster

A LARGE number of Angiosperms are characterized by the presence of ramified sclereids dispersed in a variety of patterns in the "fundamental tissue system" of the foliage leaf. In some cases, as in the petiole of *Camellia* (Foster, 1944), the sclereids occur within rather compact tissue. But very commonly, these bizarre elements are found in the midst of highly lacunate tissue with their branches extending freely into the intercellular spaces. Classical examples of the latter condition are provided by the sclereids of certain aroids (van Tieghem, 1866) and water lilies (Gürtler, 1905). Although considerable attention has been paid to sclerenchymatous idioblasts from the standpoint of their diagnostic value in systematic anatomy (Solereider, 1908; Bailey and Nast, 1944, 1945; Foster, 1944, 1945), the many fundamental problems posed by their ontogeny have received only meager consideration. Prominent among such problems is the question of the intercellular relationships which occur during the enlargement and ramification of the sclereid. Does "gliding growth" occur, in the sense of a literal "slip" between the extending arms of the idioblast and the

walls of adjacent parenchymatous elements? Or, is the branching of the "intrusive type" discussed by Sinnott and Bloch (1939, 1943), in which only the growing tips wedge their way between neighboring cells? As a background for future experimental studies, data obtained by the conventional histological methods are obviously needed. In an effort to contribute to this greatly neglected aspect of plant histogenesis, a detailed ontogenetic study has been made of the foliar sclereids in *Trochodendron* and the results are set forth in the present article.

Trochodendron was selected for several reasons. First of all, the study of a wide range of material has shown that the foliar sclereids of this species are remarkably polymorphic, ranging in form from radiately-branched to fiber-like cells (Foster, 1945, plates 1-4). The intercellular relationships of developing cells of such varied form clearly provide a rich field for ontogenetic study. Secondly, preliminary investigation showed that the sclereids occur within the highly lacunate tissues of the petiole and lamina. Consequently, an opportunity is provided for investigating the relation of the early stages in sclereid ontogeny to the developing intercellular

¹ Received for publication May 21, 1945.

spaces. Lastly, information on the developmental history of the distinctive sclereids of *Trochodendron* contributes further to our enlarging knowledge of a remarkable and, in many respects, unique dicotyledon (Bailey and Nast, 1945; Smith, 1945).

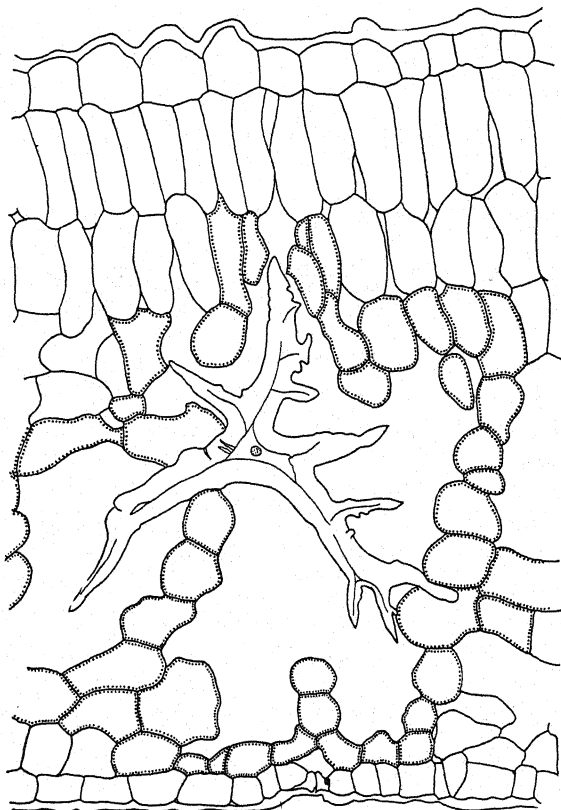


Fig. 1. Camera lucida drawing of a portion of a trans-section through the mature lamina showing the intercellular relationships of a large branched sclereid in the lacunate spongy parenchyma. A small nucleus (stippled) is present in the central lumen of the sclereid and several canal-like pits are evident in the thick, secondary wall. Parenchyma cells adjacent to the sclereid are conventionally distinguished from air spaces by means of stippling just within the cell walls. $\times 230$.

MATERIAL AND TECHNIQUE.—The material was collected from the same plant used in a previous investigation (Foster, 1945) and acknowledgment is made to Mr. Eric Walther for his generous cooperation. Foliage leaves in various stages of development up to maturity were collected from expanding shoots in late May and early June of 1944. To secure rapid penetration of the killing fluids, petioles and laminae were subdivided into small portions and the air removed by means of an aspirator. Formalin—acetic acid—ethyl alcohol (Sass, 1940, p. 16) gave satisfactory results for adult leaf tissue but caused severe shrinkage of the protoplasts and delicate primary walls of young sclereids. Good fixation of this material was obtained by using several types of CRAF killing fluids, the most successful of which

was made according to formula III as given by Sass (1940, p. 19, table 2). Dehydration, clearing with xylene and infiltration with paraffin were performed by the general procedure recommended by Ball (1941). Serial trans- and longisections of the petiole and trans- and paradermal sections of the lamina were cut eight to ten microns in thickness and stained by using tannic acid-iron chloride in conjunction with safranin (Foster, 1934). As in the case of the petiole of *Camellia*, it proved necessary to soften imbedded pieces of mature leaf tissue of *Trochodendron* by using a solution of glycerine-alcohol (cf. Foster, 1944, p. 303).

Grateful acknowledgment is made to my wife, Helen Vincent Foster, for her assistance with the illustrations used in this article.

INTERCELLULAR RELATIONS OF THE ADULT SCLEREIDS.—Before describing the origin and development of the sclereids, it is necessary to explain briefly their position and intercellular relations in the mature foliage leaf. With reference to their general distribution and form, little need be added to the account given in a recent paper (Foster, 1945). In both the petiole and lamina, the sclereids appear as idioblasts in the midst of highly lacunate parenchyma. Sclereids occur at various levels throughout the spongy parenchyma but have never been observed within the two-layered palisade parenchyma (fig. 1). Likewise in the petiole, the sclereids are confined to the inner lacunate cortex² and, in marked contrast with the situation in *Camellia*, are absent from the relatively compact, collenchymatous hypodermal parenchyma (fig. 18).

Serial sections clearly show that many of the radiating, spiculate arms of the sclereids lie freely within large intercellular spaces. But it is also evident that certain of the branches have grown between neighboring parenchyma cells and that their tips and spicules are imbedded in the tissue bordering the air lacunae (fig. 1, 26). One additional relationship must be mentioned, viz.: the presence in many sclereids of one or more vertical arms which extend from the central body of the cell toward one or both epidermal layers. This situation is particularly interesting in the rather frequent cases when one or more vertical branches protrude into the layers of palisade parenchyma. In no instance however have vertical or oblique branches been observed in direct contact with the inner tangential walls of the epidermal cells.

ORIGIN AND STRUCTURE OF SCLEREID INITIALS.—The most satisfactory cytological fixation was obtained with laminar portions cut from the "soft" and still expanding leaves of elongating shoots. Serial sections of such material reveal that although sclereids in well-advanced stages of development are present (fig. 17), new initials are also originating at various levels in the young spongy parenchyma (fig. 20, upper left). In other words, the origin

² Cf. Bailey and Nast (1945, plate 1, fig. 4-5) for illustrations of the general anatomy of the petiole of *Trochodendron*.

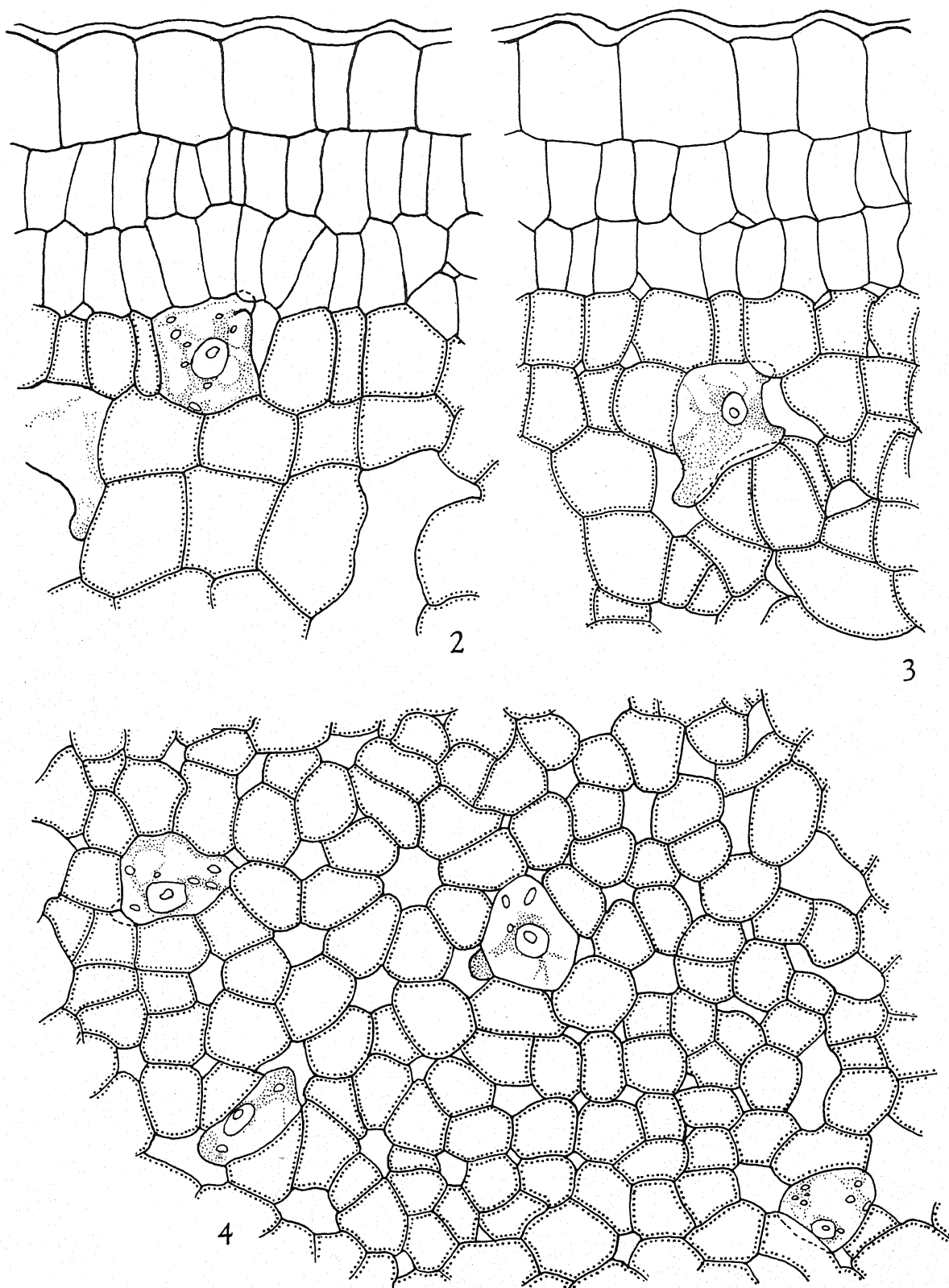


Fig. 2, 3, 4.

(See page 463 for complete legends for fig. 2 to 24.)

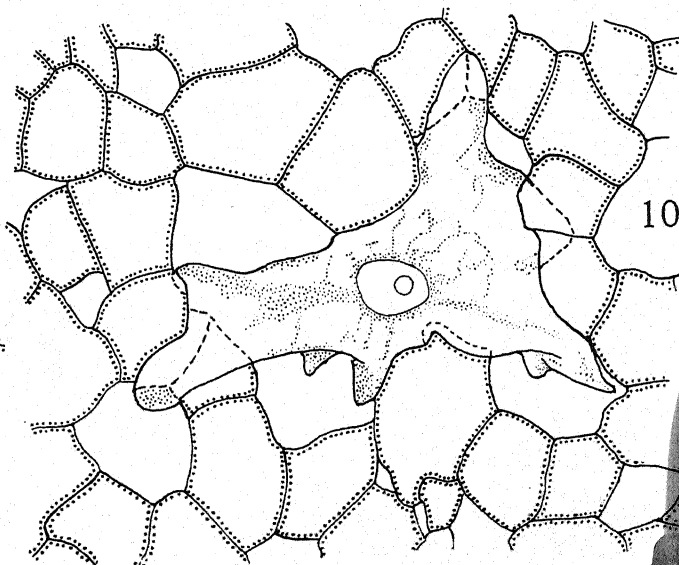
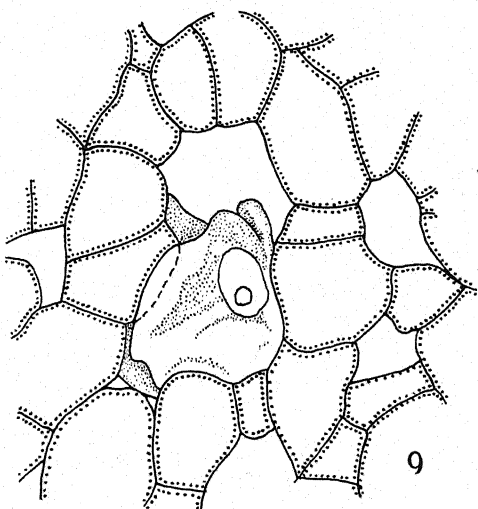
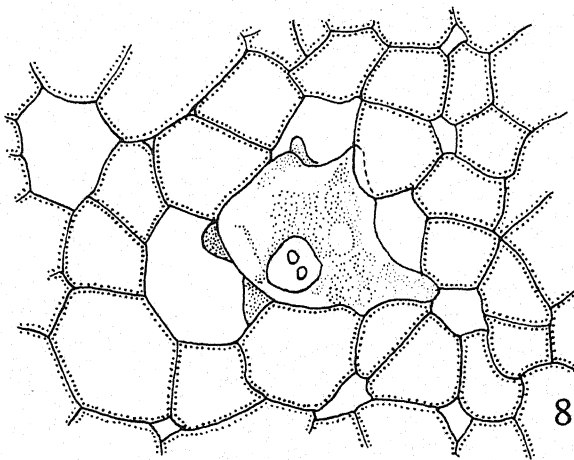
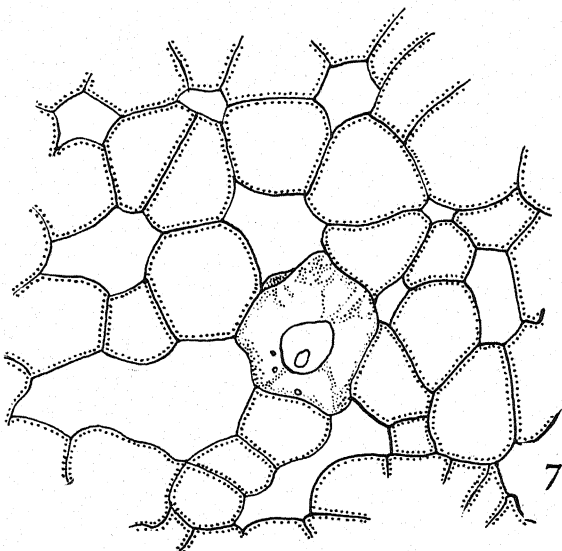
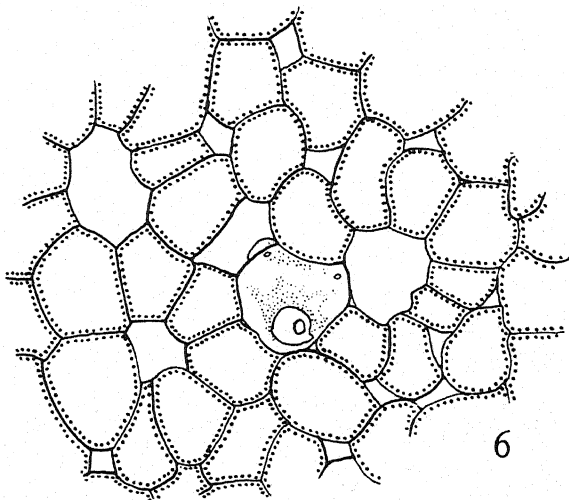
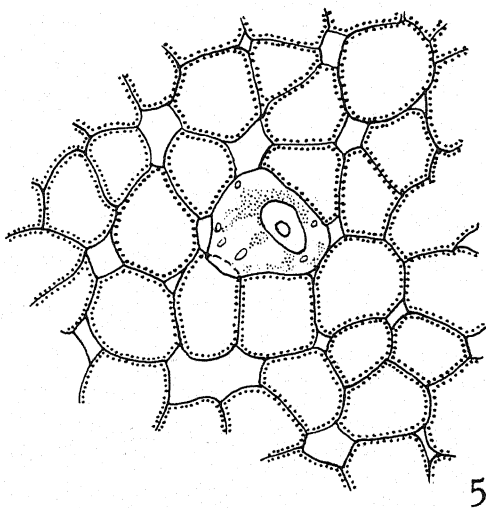


Fig. 5-10.

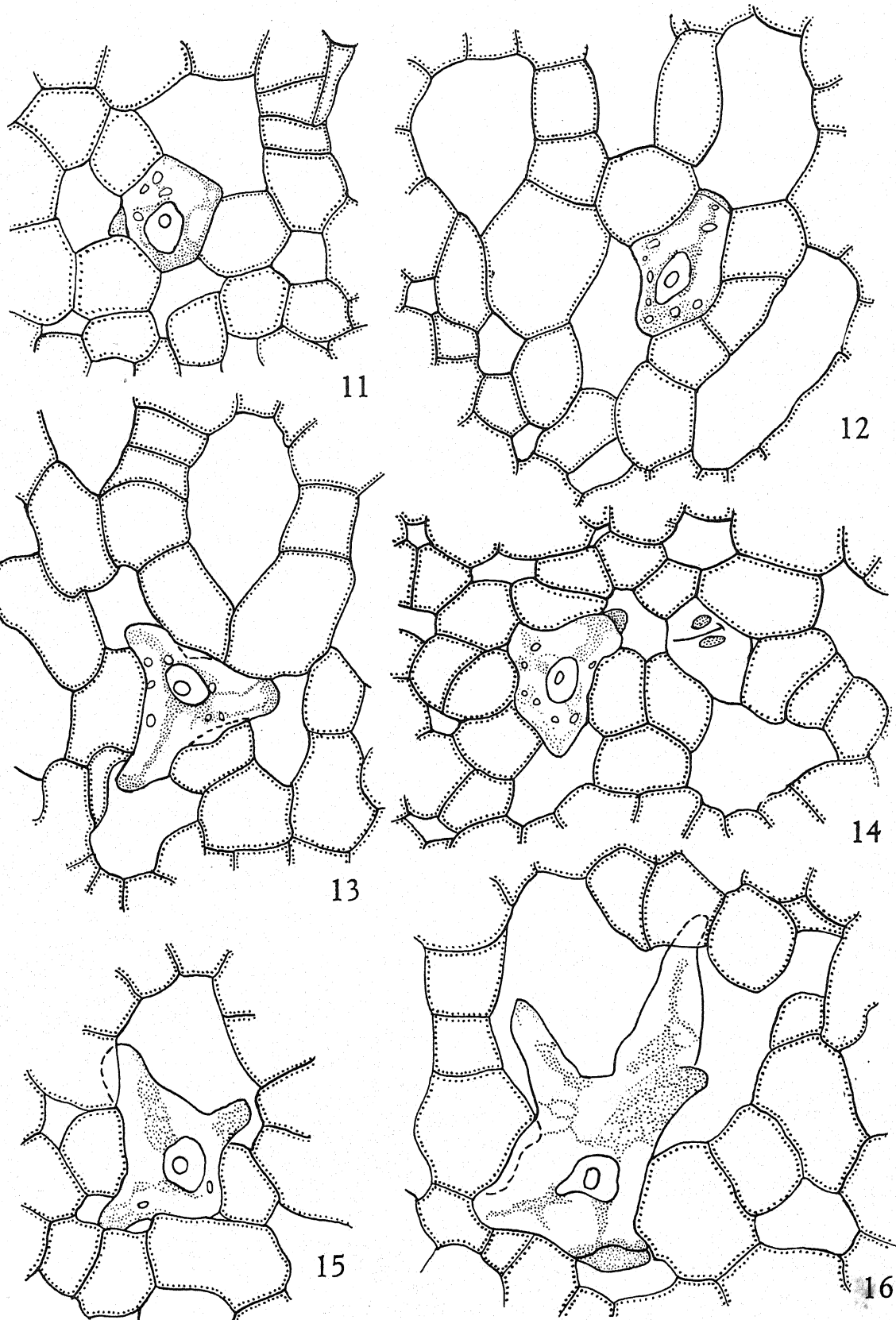


Fig. 11-16.

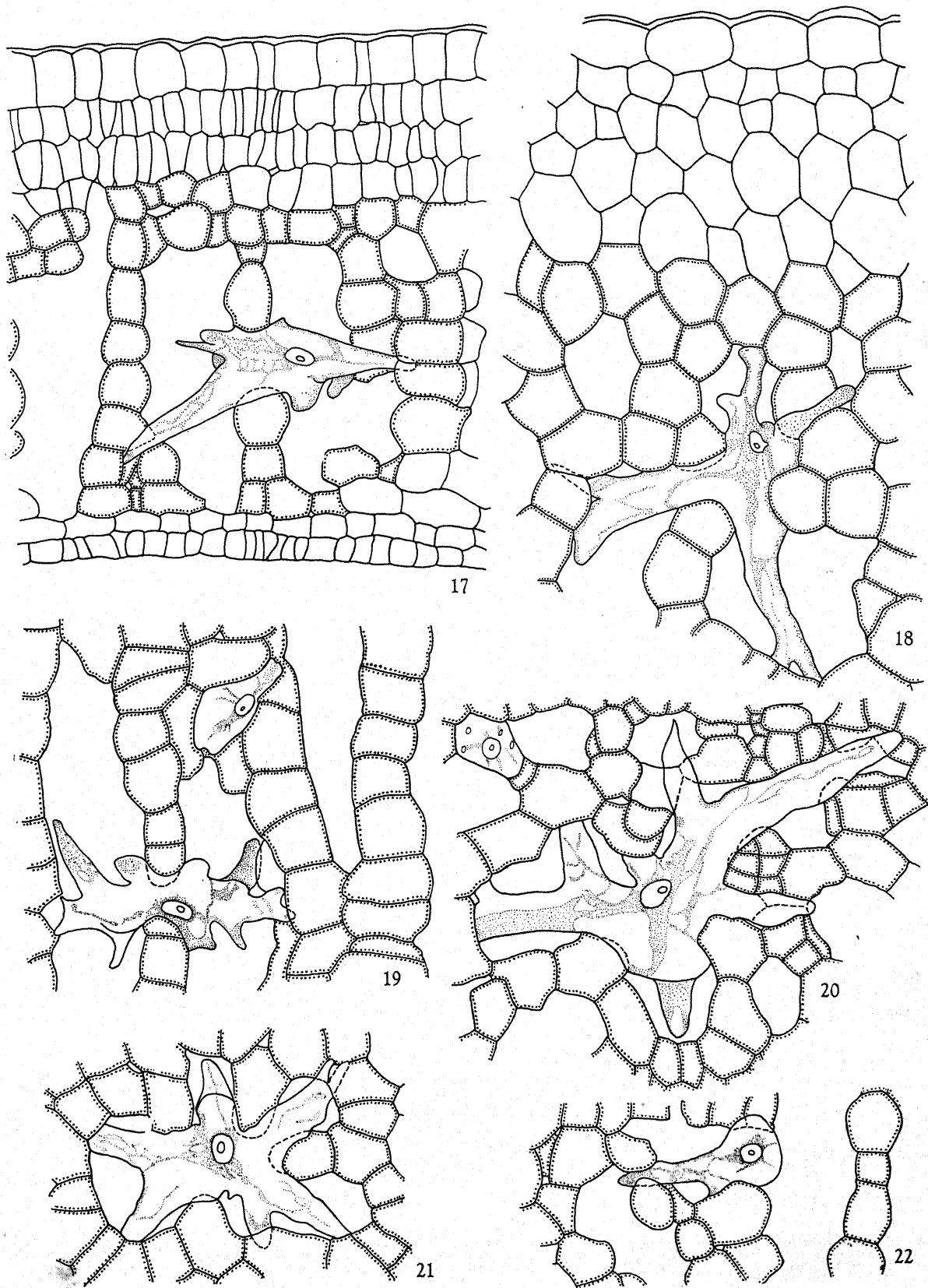


Fig. 17-22.

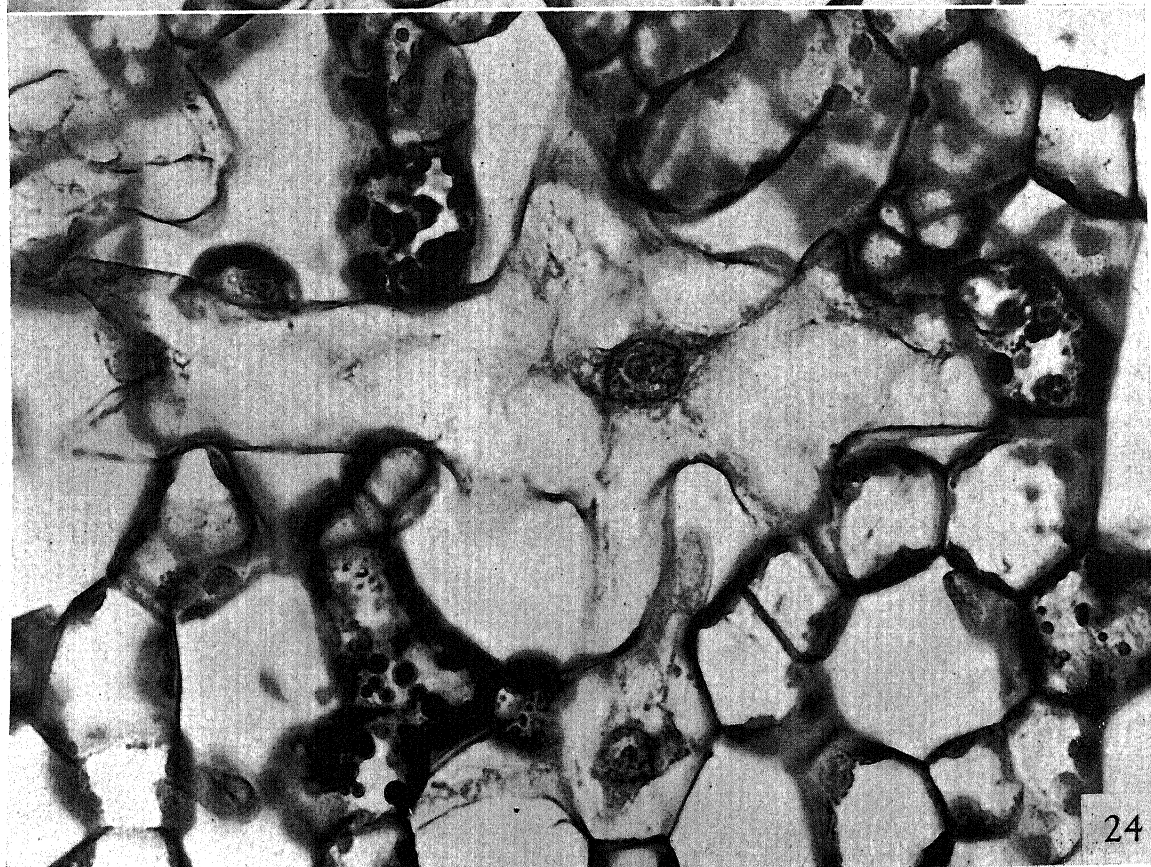
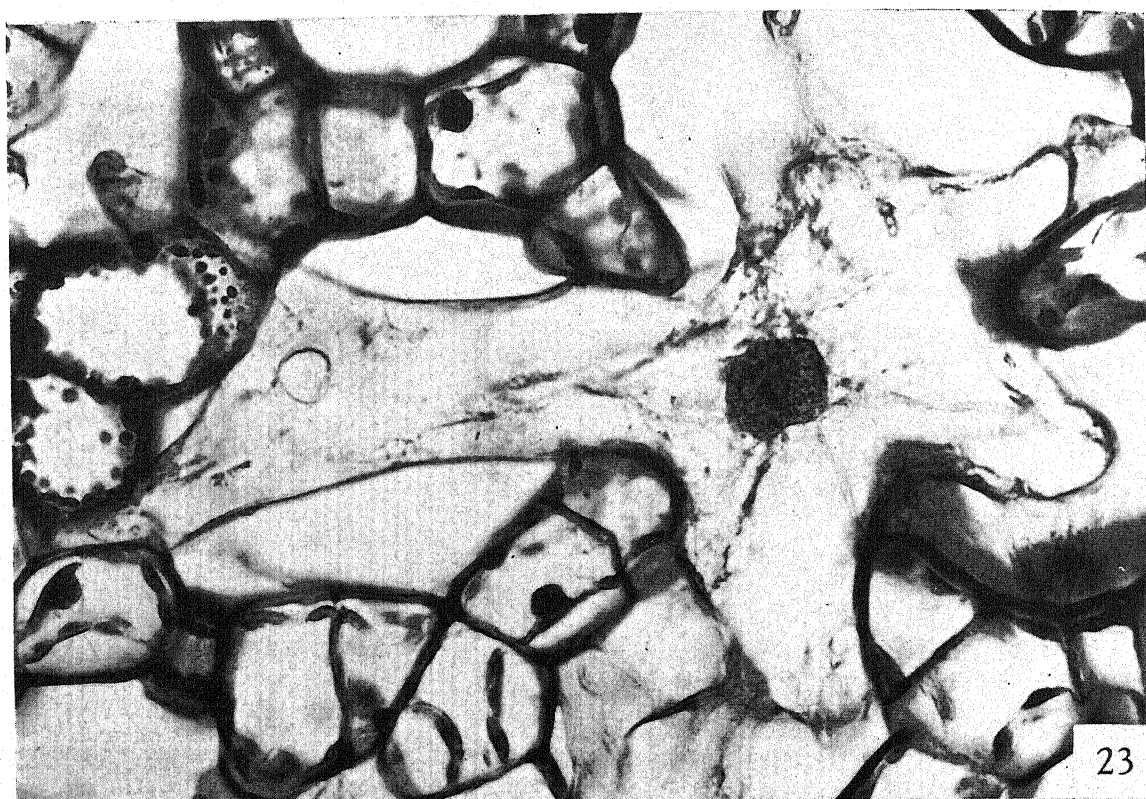


Fig. 23-24.

of laminar sclereids is not limited to the early phases of tissue maturation but occurs over a relatively extended period of time. As a result, the "cellular environment" under which the sclereids begin development varies widely, particularly with respect to the relative size of the neighboring air spaces (fig. 4, 5, 6, 11, 12, 24).

Regardless of its position in the spongy parenchyma, the sclereid initial is a relatively small polyhedral cell which is distinguished from neighboring tissue elements by the larger size of its nucleus (fig. 2, 4, 5, 6, 11, 12, 24). In other respects, e.g., general shape, thin wall and the presence of small plastids, the initial closely resembles a young mesophyll cell.

The relation of sclereid initials to adjacent cells and to the developing air-space system is clearly shown by a comparison of trans- and paradermal sections cut through the adaxial region of the young spongy parenchyma. As is shown in figures 2 and 17, this region consists of two distinct layers of cells.

In transectional view, the sclereid initials appear as isolated polygonal cells, in tight contact with the cells lying above and below them but separated from their neighbors in the same layer by small irregular air spaces (fig. 2). Paradermal sections are particularly instructive since they reveal all the lateral contacts between the initial and its neighbors and moreover illustrate clearly the complications produced by the developing intercellular spaces. Figure 5, for example, illustrates a typical situation in which the sclereid has lost certain of its original lateral contacts through the development of small intercellular spaces. At first these spaces bordering upon the sclereid initial are isolated from one another and appear triangular or quadrangular in sectional view. As development continues, however, adjacent spaces enlarge and become confluent, not only in the vicinity of young sclereids but throughout the layer of mesophyll cells. As a consequence, the size and shape of the intercellular spaces as seen in an extensive para-

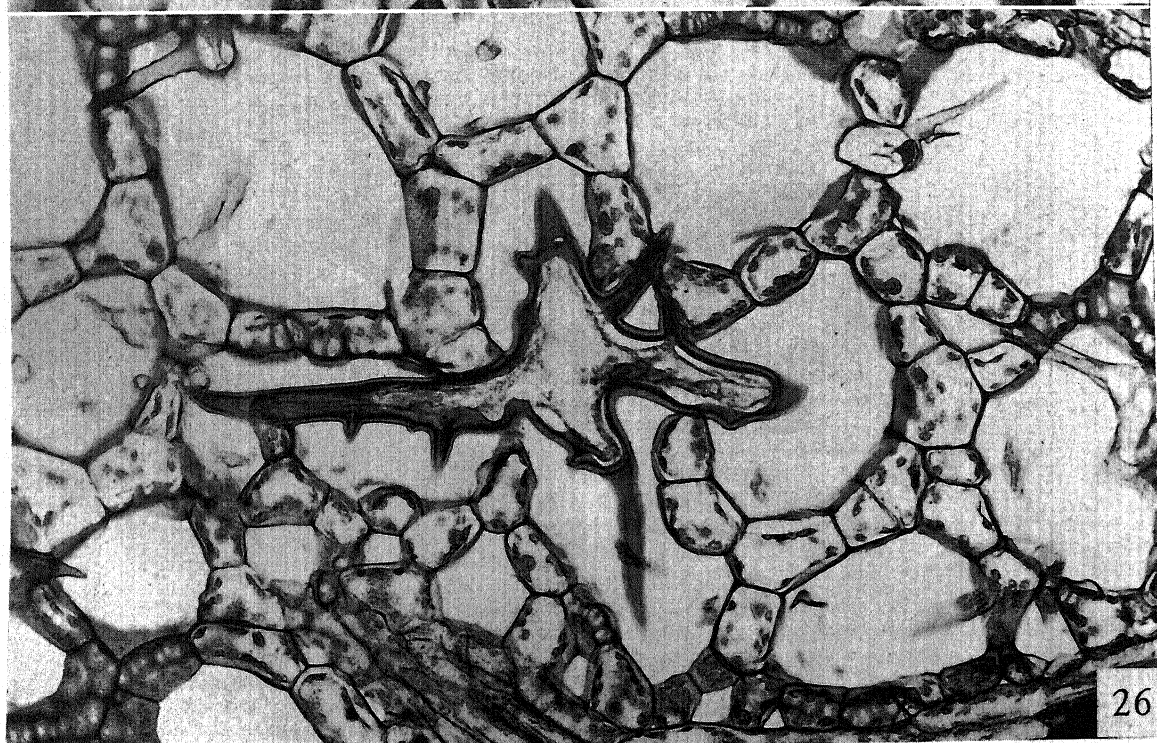
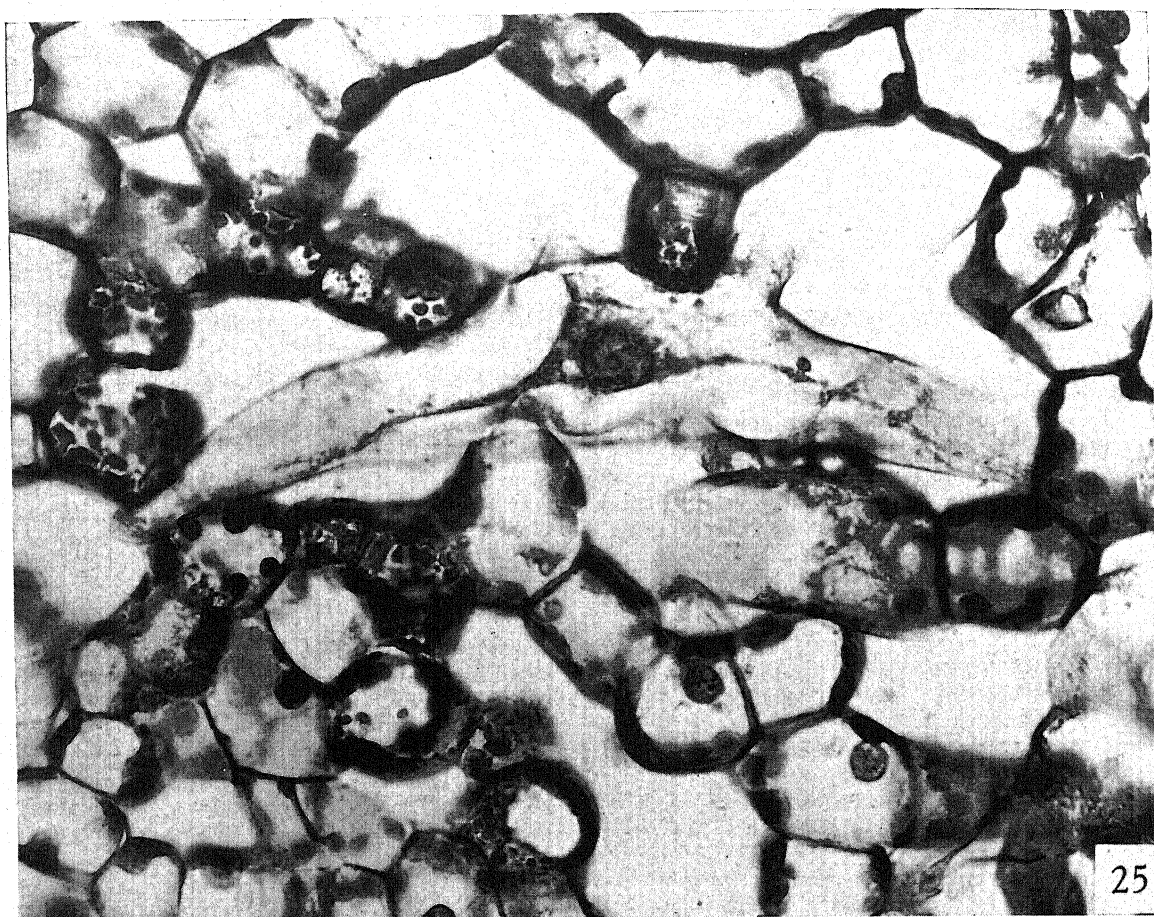
Fig. 2-4. Camera lucida drawings of sections through the adaxial region of expanding leaf blades illustrating early stages in sclereid development. Spongy parenchyma cells are conventionally distinguished from air spaces by stippling just within the cell walls. Fig. 2-3, transections, Fig. 4, paradermal section.—Fig. 2. Young sclereid beginning development in contact with inner of two layers of young palisade parenchyma.—Fig. 3. Young sclereid with three embryonic branches protruding into adjacent air spaces.—Fig. 4. Intercellular relations of four young sclereids developing within layer of embryonic spongy parenchyma. Note great variety in size and shape of intercellular spaces. $\times 720$.

Fig. 5-10. Camera lucida drawings of paradermal sections through the embryonic layer of spongy parenchyma illustrating early stages in sclereid ontogeny. Parenchyma cells are conventionally distinguished from air spaces by stippling just within the cell walls.—Fig. 5-6. Sclereid initials. Note variation in size of adjacent intercellular spaces in the two figures.—Fig. 7. A young sclereid with papillate branch-primordia extending into neighboring air spaces.—Fig. 8. More advanced stage in branching. Note that the paired branch primordia at the top and left of the sclereid are at different levels.—Fig. 9. The invasion of an air space by a trio of branch primordia.—Fig. 10. The amoeboid appearance typical of a more advanced stage in development. Note that two of the larger branches have traversed neighboring air spaces and are extending between cells in their path. $\times 720$.

Fig. 11-16. Camera lucida drawings of paradermal sections through the lacunate abaxial region of the developing spongy parenchyma illustrating early stages in sclereid ontogeny. Parenchyma cells are conventionally distinguished from air spaces by stippling just within the cell walls.—Fig. 11-12. Sclereid initials, showing variations in their sectional form and their relationships to adjacent cells and air spaces.—Fig. 13. A young sclereid with three branches radiating into adjacent air spaces.—Fig. 14. An early stage in branching. Note that the curved cell-plate in the dividing cell at the right of the sclereid is oriented perpendicularly to the adjacent air space. Divisions of this type produce circles of cells which enclose polygonal air spaces.—Fig. 15-16. Two stages of dichotomous branching within prominent air chambers. Near the base of the larger of the two branches of the sclereid in figure 16, a new branch primordium has originated. $\times 720$.

Fig. 17-22. Camera lucida drawings of sections illustrating advanced stages in sclereid ontogeny. Parenchyma cells adjacent to the sclereids are conventionally distinguished from air spaces by stippling just within the cell walls.—Fig. 17. Transection of a portion of an expanding lamina showing the form and intercellular relationships of a large sclereid within the spongy parenchyma.—Fig. 18. Transection of a small portion through the epidermis and cortex of an elongating petiole, showing the intercellular development of a large sclereid within the lacunate parenchyma. Note the dichotomous branching of the tips of the two longest branches.—Fig. 19. Longisection through the lacunate parenchyma of a young petiole showing the complex branching of a sclereid within the longitudinal air chambers. A very young sclereid is seen above this cell.—Fig. 20. Paradermal section through a large sclereid in the spongy parenchyma of the lamina. Note the complex type of branching and the invasion of air spaces and new tissue by the growing arms. A sclereid initial (shown with nucleus and young plastids) is seen at the upper left of this figure.—Fig. 21. Paradermal section showing a large proportion of the sclereid branches within the adjacent air cavities.—Fig. 22. Transection of a small portion of the lacunate spongy parenchyma illustrating the growth of a sclereid branch between cells and its entrance into an air space. $\times 360$.

Fig. 23-24. Oil immersion photomicrographs of paradermal sections of advanced stages in sclereid ontogeny. Note the amoeboid appearance of the sclereids, and their large nuclei and delicate primary walls.—Fig. 23. The left branch of the sclereid has traversed a large air chamber, dichotomized and the tips have grown between cells. The upper tip is entering a new air chamber.—Fig. 24. A considerable part of the central body of the sclereid lies within a large air chamber, and the forked tip of the left branch has just entered a new lacuna. At the base of the figure, one of the delicate tubular branches of a much younger idioblast lies within the same air space occupied by the larger sclereid. $\times 800$.



dermal section vary within wide limits (fig. 4). An important factor in the continued enlargement of the intercellular spaces consists in the repeated division and enlargement of many cell groups which surround polygonal air spaces. Since the new division walls are typically inserted at right angles to the air spaces, rather well-defined circular cell groups result. A clear example of this is shown at the upper left of the central sclereid in figure 4. Not infrequently, one or more of the cells of these circular groups are sclereid initials (fig. 6) which, unlike their neighbors, soon begin to grow into the central air lacuna.

In the lower highly lacunate region of the developing spongy parenchyma, the sclereid initials are flanked by large circular or polygonal air spaces. Frequently, as seen in paradermal sections, the initial is hexagonal in shape, with three of its faces bordering upon the adjacent lacunae (fig. 11). In other cases, however, irregular divisions and enlargement in the adjacent mesophyll cells result in less symmetrical relationships (fig. 12).

INTERCELLULAR DEVELOPMENT OF SCLEREIDS.—Originating within the developing spongy parenchyma as a small polyhedral cell, the sclereid initial enlarges and begins its highly individualistic development (fig. 7-10). The difficulties of interpreting the three-dimensional form of such a growing, ramifying cell are obvious and may be solved only by the use of the wax-plate reconstruction technique which has been employed in studying the shapes of parenchyma cells (Lewis, 1944). However, the following account is based upon the careful examination of a wide series of closely-connected developmental stages and is believed to cover the salient features of sclereid ontogeny.³

The earliest phases of branching consist in the extension of small papillate protuberances into certain of the air spaces which border upon the young sclereid. It seems evident from a comparison of trans- and paradermal sections that such protuberances may begin growth nearly simultaneously at different levels from various faces of the same cell (fig. 3, 7, 8, 9). In other words, radiate branching is initiated during the very early stages of ontogeny. Since branch initiation is accompanied by a general enlargement of the sclereid, the cell soon loses its original polyhedral form and appears as an irregularly-lobed structure which is actively expanding into the neighboring air spaces (fig. 8-10, 13-16). At this stage, the relation of the young sclereid to

³ The present investigation is necessarily limited to a study of the ontogeny of the ramified type of foliar sclereid in *Trochodendron*. Data on the development of the fiber-like types described in detail in a recent article (Foster, 1945, p. 157-158) will not be available until collections of living material can be secured from Formosa.

adjacent cells is relatively clear, particularly in paradermal sections through highly lacunate regions of the spongy parenchyma. In such cases, the original cell contacts between the sclereid and its neighbors in the same cell layer are perfectly evident (fig. 13-16). But in more compact regions of the parenchyma, the original intercellular relationships soon become obscured for several reasons. First, the young mesophyll cells surrounding the sclereid are still dividing (fig. 14). Consequently, the number of cell contacts between the enlarging sclereid and adjacent tissue elements undoubtedly increases, at least for a time. In the second place, certain of the original contacts between the sclereid initial and its neighbors are lost during early growth because of the origin and enlargement of new intercellular spaces. This has undoubtedly occurred in the case of the young sclereid in the center of figure 4. Lastly, the rapidly-extending arms of the young sclereid sooner or later traverse the small intercellular spaces into which they have extended and hence come into direct contact with new tissue elements between which they continue to grow and ramify (fig. 8, 10). It is thus apparent that the form and cell contacts of a young sclereid at a given stage in ontogeny depend upon the interaction of a changing series of factors. Perhaps the most definitive of these factors is the progressive invasion of new air spaces and new tissue by the rapidly-growing arms of the sclereid (fig. 10). These later phases in ontogeny will now be described.

Because of the irregular nature of branching, sectional views of advanced stages in sclereid development present a series of widely variable cell forms. A representative transectional view is shown in figure 17. At the level of section illustrated, the main body of the sclereid is firmly in contact with the cells of a vertical plate of young parenchyma cells which are flanked on each side by prominent air lacunae. Extending into these spaces from the central body of the sclereid are a series of developing arms and protuberances, the two longest of which have traversed the spaces and grown in between certain of the cells in their path. Exceptionally clear examples of the capacity of sclereid branches to grow from one air space into an adjoining cavity through intervening tissue are depicted in figures 22 and 25.

When advanced stages in ramification are studied in paradermal sections, the sclereids appear as amoeboid structures with their lobes and dichotomizing arms often occupying a large part of the adjacent air lacunae (fig. 20, 21, 23, 24). Since the direction of growth of the radiating arms of the sclereid are often oblique with reference to the plane of section, it is impossible to trace the termination of many of the ramifications (fig. 21). But it is clear in those

Fig. 25-26.—Fig. 25. Oil immersion photomicrograph of a paradermal section of a large sclereid illustrating very clearly, at the left, the tip of one arm growing between cells and entering an air chamber. The small scattered discoid bodies in the cytoplasm of this sclereid presumably are plastids. $\times 800$.—Fig. 26. Photomicrograph of a paradermal section through the spongy parenchyma of a fully-expanded lamina showing the early stages in formation of the secondary wall of a large sclereid. The nucleus is seen next to the wall at the right. Above and to the right of this cell, a young sclereid has sent out a delicate tubular branch into an adjacent lacuna. $\times 325$.

cases where a portion of the branches lie in the horizontal plane that complex and widely-fluctuating intercellular relationships arise. Often a given arm forks or even becomes trifurcate during its early growth within an air space (fig. 10, 16). When such a dichotomous branch in its further growth reaches a cellular partition, one of the alternatives is followed, viz.: (1) both of the tips may grow at the same level between the cells in their path and enter a new air space (fig. 24), or (2) the tips may diverge in the direction of their growth, each one entering a separate air space or else invading additional tissue (fig. 20). Successive stages in the second possible method of growth are illustrated by figures 10 and 23.

In highly lacunate portions of the spongy parenchyma the arms of several different separated sclereids may often be seen within the same lacuna. An interesting example of one of the ways in which this condition arises is shown in figure 24. Near the lower edge of the photomicrograph a delicate tubular branch of a young sclereid is seen extending into the same air space as that partly occupied by certain of the ramifications of the large radiately-branched sclereid. Many examples were found where the lobes and branches of different sclereids appeared to be interlocked. Whether, in these cases literal "cell fusion" occurs, as a result of the breakdown of the tenuous walls, is difficult to determine. No convincing evidence has been found however to support such a possibility.

Although satisfactory fixation of developing sclereids in the petiole was less frequent than in the lamina, the general program of development appears fundamentally similar in both regions of the foliage leaf. In transections of the petiole, the growing sclereid appears as a delicate amoeboid cell with its tubular branches extending across the prominent air chambers and penetrating adjacent cellular partitions (fig. 18). Longitudinal sections often reveal an extensive and complex series of vertical and oblique branch primordia developing freely within the longitudinally extended air spaces. In the cell shown in figure 19, continued vertical elongation of the larger branch primordia in each air chamber in opposite directions would produce the characteristic H-shaped petiolar sclereids described and figured in a previous paper (Foster, 1945, plate 2, fig. 3).

In concluding this section, certain of the cytological features of the growing sclereid deserve emphasis. First of all, the distinctively large size of the nucleus is maintained throughout the period of enlargement and ramification. In agreement with the petiolar sclereids of *Camellia* (Foster, 1944, p. 311-313), the sclereids of *Trochodendron* show no evidence of nuclear division at any stage in sclereid ontogeny. Although in fixed material the nucleus assumes a variety of positions in the central body of the sclereid, it has not been observed in the more distal portions of the lobes or in the branches. The cytoplasm is evidently highly vacuolated and closely resembles in its appearance the delicate cyto-

plasm of plant hairs (fig. 23-25). Small discoid bodies, which appear to be plastids, may occur even in advanced stages of sclereid ontogeny (fig. 25). But on the basis of the fixation images examined in this investigation, there is good reason to believe that at least the majority of the plastids which are present in sclereid initials degenerate as the cell enlarges and branches.

SPICULES AND SECONDARY WALL FORMATION.—One of the distinctive features of the adult sclereid in *Trochodendron* is the presence of small spicules on the arms as well as on portions of the central body. These local outgrowths of the wall fluctuate widely in their number and distribution (Foster, 1945, plate 1-3). Because of the oblique and undulating course of the arms of developing sclereids, it has proved difficult to determine the order of appearance (if any) of the spicules. As is shown in figure 27, spicules may originate as small irregularly distributed protuberances from the delicate primary wall of branches which lie freely within air spaces. Spicules also develop at the points of contact between a sclereid branch and parenchyma cells (fig. 28). But the sequence of formation of spicules appears highly variable and is not clearly related to the immediate environment of a given sclereid.

After the sclereid has completed its phase of enlargement and ramification, a thick secondary wall is deposited by the protoplast (fig. 26). Centripetal thickening of this wall may proceed to such an extent that, except in the central body, the lumen is reduced to a narrow canal-like cavity. In marked contrast to the very abundant canal-like pits typical of the sclereids in *Camellia* (Foster, 1944, fig. 19-32), pits are sparsely distributed in the sclereids of *Trochodendron* and appear restricted to the central body of the cell (fig. 1). Although a detailed investigation of their development and structure was not attempted, studies made with the oil immersion lens show that they are paired with the delicate pit-fields of contiguous parenchyma cells. Occasional examples of ramiform pits were seen although much less commonly than in *Camellia*.

DISCUSSION.—The developmental history of the branched sclereids of *Trochodendron* raises many questions of general histogenetic interest, the most significant of which now may be discussed.

The young sclereid, in marked contrast to the parenchymatous cells surrounding it, pursues a highly individualistic path of development. Its radiating branches enter adjacent air spaces, traverse them and grow between the cell walls of compact tissue masses. As a result, new cell contacts are made progressively during the ontogeny of the sclereid. Obviously the intercellular relationships which result from such a remarkable type of cell development are both complex and variable. If the sclereid begins its existence as a cell in the highly lacunate region of the spongy parenchyma, a very considerable part of its ramifying growth occurs within the air-space system (fig. 20-21). But sclereid initials also arise in regions of more compact spongy paren-

chyma (fig. 2, 5). In this case, the branches soon encounter tightly-joined cells between which they extend and enlarge (fig. 8, 10). The conclusion, therefore, seems justified that the sclereid is an "opportunist" in response to its immediate environment, taking full advantage of any air spaces which may adjoin it but also possessing the capacity for growth between tissue elements in its path (fig. 17, 18, 20, 22-25). From this standpoint there appears to exist no fundamental ontogenetic difference between the petiolar sclereids of *Camellia* (Foster, 1944) which arise and branch within relatively compact tissue, and the foliar sclereids of *Trochodendron*. Evidently prominent air spaces in the immediate vicinity of a developing sclereid may "favor" radiate branching, but they do not appear to represent the primary factor determining cell form. On the contrary, the capacity of a sclereid to ramify must depend fundamentally upon a sustained and aggressive growth of its primary wall not shared by neighboring parenchyma elements. This idea leads to the question posed in the introduction of this paper, i.e., does "gliding growth" occur during the intercellular branching of the sclereid?

As was pointed out in a recent paper (Foster, 1944, p. 319-320), the "mechanics" which underlie the extension of the arms of a developing sclereid between neighboring tissue elements are far from clear. Küster (1925, p. 316-318) adopts the concept of "gliding growth," whereas Sinnott and Bloch (1943, p. 98) and Bloch (1944, p. 75) favor the idea of "intrusive growth." Convincing evidence in support of either of these theories must come ultimately from the direct observation of cell growth as well as from a better understanding of the nature of cell-wall development (van Wisselingh, 1925, p. 210-232). Nevertheless, the fact that spicules originate at various points on the arms of developing sclereids does not support the assumption of the theory of "intrusive growth" that the extension of the wall is restricted to the very tips of the branches. On the contrary, spicule development appears to reflect the highly plastic nature of considerable portions of the primary wall during the ramifying growth of the sclereid (fig. 27-28). Therefore "movement" of the sclereid wall along the surfaces of cells bordering on air chambers as well as between the walls of tissue elements is entirely possible although by no means proved.

In criticizing the concept of "gliding growth" as applied to cambium cells and non-articulated latex ducts, Sinnott and Bloch (1939, p. 632) maintain that "there is no evidence that the surface of one cell slides over another during the process. The growth of the intruding cell may be thought of as that of a root hair moving through the soil by apical growth or of a tylosis-like structure which pushes out into a mass of tissue instead of into a cavity." Whether these analogies are applicable to the remarkable development of the sclerenchymatous idioblasts discussed in the present article is an open question. The writer, however, cannot agree with

Sinnott and Bloch's (1939, p. 633) statement that in the case of intrusive growth "the development of such a system could be more readily explained on the assumption of a mechanism inherent in the whole cell complex and not related to the behavior of independent units within it" (italics by the present writer). If applied to the developing spongy parenchyma of *Trochodendron*, this assumption seems to the writer to oversimplify a complex situation. The entire career of development of an idioblastic sclereid

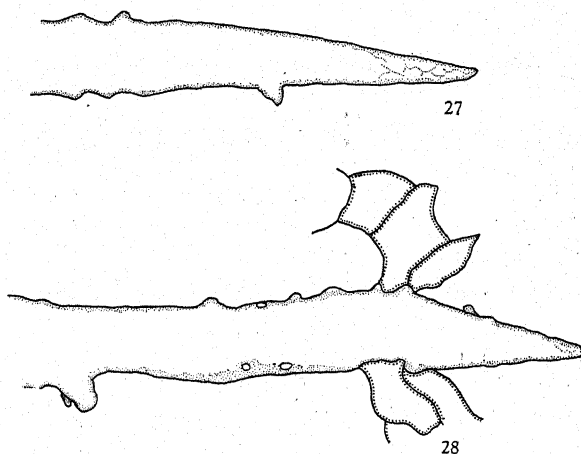


Fig. 27-28. Camera lucida drawings made under oil immersion illustrating the irregular development of spicules from the growing primary wall of sclereid branches. —Fig. 27. Developing spicules on a branch lying freely in an air chamber. —Fig. 28. Spicules developing on a branch at its point of contact with parenchyma as well as on portions lying within the adjacent air chambers. Both figures $\times 230$.

reid is marked by a "rugged individualism" which suggests that the normal correlative forces operative during the differentiation *en masse* of parenchyma elements have been replaced locally by new and unique factors. To assess the nature of such factors lies beyond the scope of histogenetic investigation. It is doubtless significant however that all of the spongy parenchyma cells appear to retain the capacity to enlarge and to ramify throughout the period of maturation of the lamina. This is clearly illustrated in figure 26 which represents a paradermal section through the spongy parenchyma of a fully-expanded leaf blade. Above and to the right of the large thick-walled sclereid, a young idioblast has sent out a delicate tubular branch into the adjacent air chamber. Many examples of such tardy development of sclereids have been observed in the present study, emphasizing the sustained capacity of parenchymatous elements for individualistic growth at various stages in organ ontogeny (Foster, 1942, p. 58-59; Bloch, 1944). It is to be hoped that future experimental technique may reveal the physiological factors which induce the idioblastic development of certain cells while inhibiting the further growth of their neighbors.

SUMMARY

The branched sclereids typical of the mature foliage leaf of *Trochodendron* occur as idioblasts dispersed within the lacunate parenchyma of the petiole and the spongy parenchyma of the lamina. This paper describes their origin and development.

The lamina sclereid originates from a small, polyhedral cell which closely resembles a young mesophyll cell except for the larger size of its nucleus. The "cellular environment" under which a sclereid initial begins to enlarge and to branch varies widely. In the highly lacunate spongy parenchyma of expanding leaf blades, the initial borders upon large air chambers. But in more embryonic portions of the mesophyll, the young sclereid is separated from some of its neighbors only by small triangular or quadrangular air spaces.

Radiate branching of the sclereid initial results from the extension of small papillate protuberances into certain of the adjacent air spaces. The initiation of branching is accompanied by an enlargement of the sclereid and the loss of its original polyhedral form. As development continues, the extending arms of the sclereid traverse the neighboring air spaces and grow between the walls of compact tissue in their path. In advanced stages of branching, the sclereid appears as an amoeboid structure with its lobes and arms related in a variety of ways to the air spaces and tissue in its vicinity. Often the arms of separate sclereids may occur, more or less interlocked, within the same air chamber.

The general program of development of petiolar sclereids is essentially similar to that in the lamina. Extensive vertical elongation of certain branches of the sclereid within adjacent longitudinally-extended air chambers gives rise to the H-shaped type of sclereid commonly found in the petiole of *Trochodendron*.

The sclereid, throughout its active period of branching, retains its single large nucleus. There is evidence that the small plastids typical of sclereid initials tend to degenerate during the later phases of ontogeny.

Spicules originate as small outgrowths from the primary wall of the sclereid. There appears to be no fixed sequence in the order of their initiation on a given sclereid arm. When enlargement and ramification have ceased, the sclereid forms a massive secondary wall provided with sparse canal-like pits.

It is concluded that the presence of air spaces bordering a given sclereid initial is not the primary factor inducing cell branching and hence specific form. On the contrary, the primary wall of the young sclereid possesses an unique capacity for sustained and aggressive growth not shared by neighboring parenchyma cells.

A brief discussion is given of the concepts of "gliding" and "intrusive" growth as applied to the intercellular development of branched sclereids.

DEPARTMENT OF BOTANY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

- BAILEY, I. W., AND C. G. NAST. 1944. The comparative morphology of the Winteraceae, V. Foliar epidermis and sclerenchyma. *Jour. Arnold Arboretum* 25: 342-348.
- , AND ———. 1945. Morphology and relationships of *Trochodendron* and *Tetracentron*, I. Stem, root and leaf. *Jour. Arnold Arboretum* 26: 143-154.
- BALL, E. 1941. Microtechnique for the shoot apex. *Amer. Jour. Bot.* 28: 233-243.
- BLOCH, R. 1944. Developmental potency, differentiation and pattern in meristems of *Monstera deliciosa*. *Amer. Jour. Bot.* 31: 71-77.
- FOSTER, A. S. 1934. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. *Stain Tech.* 9: 91-92.
- . 1942. Practical plant anatomy. Van Nostrand, New York.
- . 1944. Structure and development of sclereids in the petiole of *Camellia japonica* L. *Bull. Torrey Bot. Club* 71: 302-326.
- . 1945. The foliar sclereids of *Trochodendron aralioides* Sieb. & Zucc. *Jour. Arnold Arboretum* 26: 155-162.
- GÜRTLER, F. 1905. Ueber interzelluläre Haarbildungen, insbesondere über die sogenannten inneren Haare der Nymphaeaceen und Menyanthoideen, Inaug. -Diss. Berlin.
- KÜSTER, E. 1925. Pathologische Pflanzenanatomie. Dritte Auflage. Jena.
- LEWIS, F. T. 1944. The geometry of growth and cell division in columnar parenchyma. *Amer. Jour. Bot.* 31: 619-629.
- SASS, J. E. 1940. Elements of botanical microtechnique. McGraw-Hill, New York.
- SINNOTT, E. W., AND R. BLOCH. 1939. Changes in intercellular relationships during the growth and differentiation of living plant tissues. *Amer. Jour. Bot.* 26: 625-634.
- , AND ———. 1943. Development of the fibrous net in the fruit of various races of *Luffa cylindrica*. *Bot. Gaz.* 105: 90-99.
- SMITH, A. C. 1945. A taxonomic review of *Trochodendron* and *Tetracentron*. *Jour. Arnold Arboretum* 26: 123-142.
- SOLEREDER, H. 1908. Systematic anatomy of the dicotyledons. Vol. 2, p. 1090-1092.
- VAN TIEGHEM, P. 1866. Recherches sur la structure des Aroideés. *Ann. Sci. Nat. Bot. Sér. 5.* 6: 72-210.
- VAN WISSELINGH, C. 1924. Die Zellmembran. *Handb. Pflanzenanatomie* Bd. III/2 I Abt. 1 Teil.

PLANT GROWTH UNDER CONTROLLED CONDITIONS. V. THE RELATION BETWEEN AGE, LIGHT, VARIETY AND THERMOPERIODICITY OF TOMATOES ¹

F. W. Went

IN AN earlier paper (Went, 1944a) the conclusion was reached that tomato growth responded more to temperature than to humidity of the air, length of illumination, light intensity, or nutrition. Slight differences in night temperature caused considerable difference in rate of stem elongation or in fruit set. This work has been continued, and in the present paper a closer analysis of the temperature effect is given, and the temperature response of a number of tomato varieties is described. In the next paper of the series (Went and Cosper, 1945) these data will be compared with the development of these same varieties under practical conditions in the field.

In all the following experiments, except where the contrary is stated, tomato seeds were germinated in sand in an ordinary greenhouse, and the seedlings were watered with nutrient solution. After the first foliage leaves had fully developed, the plants were transferred to one-gallon crocks filled with crushed rock, and they were left outside in a lath house for about two weeks. Then the temperature treatments were started.

More different night temperatures could be tested simultaneously than in the previous experiments, since a few incubators were built into the dark rooms, which were kept at 4°C. above the darkroom temperature. They were not quite satisfactory and the rates of stem elongation did not quite reach those expected. In total weight and fruit weight, however, the values at 22° in the incubator were intermediate between the higher and lower temperatures.

¹ Received for publication May 28, 1945.

RELATION BETWEEN AGE, TEMPERATURE AND STEM GROWTH RATE.—In a previous article (Went, 1944c) it was concluded that the decrease in growth rate of full-grown tomato plants (variety San Jose Canner) when subjected to night temperatures above 17–18°C. was due to insufficient translocation of sugars from the leaves at these higher temperatures. It also was pointed out that the higher optimum night temperature encountered in seedlings could be explained by their small size so that translocation was less critical than in large plants. If this were actually true, one would expect a *gradual* lowering of the optimal night temperature as the tomato plant grew, and this was tested.

A group of tomatoes (San Jose Canner) were divided into four groups: two were grown at 26°C. during the day and two at 18° during the day. In each of these temperature conditions one group consisted of plants 250–280 mm. long and grown in one-gallon crocks in crushed rock, and the other group were 60–90 mm. tall, grown in four-inch pots in sand. All were watered twice daily with nutrient solution. One-sixth of each of these groups were placed at six different temperatures each night from 16:00 to 8:00. Measurements of stem length were taken every two days. Tables 1 and 2 and figure 1 show the results of these measurements. From table 1 it appears that the steady growth rate at a constant day and night temperature of 26°C., after an initial spurt to 29 mm./day, has settled down to 24.7 mm./day in the period May 1–15. This rate is slightly above the average rate of 23.1 as found in earlier

TABLE 1. Growth rate in mm./day for San Jose Canner tomatoes grown in one-gallon crocks at different day and night temperatures.

Day temperature Night temperature	26.5°C.						18°C.						Growth at 30° as % of that at 16°C.	Weather conditions
	30	26.5	22	16	13	8	30	26.5	22	16	13	8		
April 19–22	12.0	15.3	8.6	8.5	6.3	1.8	13.1	6.1	8.9	4.3	3.5	1.9	196	
April 22–25	17.9	18.9	16.4	14.7	10.5	3.8	15.7	12.0	8.8	6.1	4.1	1.7	163	
April 25–27	19.6	25.8	22.3	16.8	12.4	3.4	17.1	15.2	12.9	7.7	6.6	2.1	150	Overcast, hazy
April 27–29	21.4	29.6	22.6	17.0	14.8	6.1	15.4	15.5	13.3	8.9	6.8	2.3	142	Overcast, hazy, rain
April 29–May 1	23.2	27.2	22.0	23.6	16.5	3.3	18.5	19.2	17.1	13.7	9.9	2.4	112	Broken clouds, hazy
May 1–3	20.8	24.1	25.5	26.3	16.8	4.3	13.5	15.5	13.0	12.0	9.0	2.5	90	Clear-overcast, hazy
May 3–5	20.8	23.5	22.9	27.6	18.1	5.2	18.3	17.3	14.1	13.8	9.2	3.3	94	Clear
May 5–7	21.8	25.1	23.5	26.6	21.8	6.5	18.3	19.6	16.0	17.3	10.4	3.4	91	Clear
May 7–9	21.9	28.0	24.5	30.0	19.5	5.4	15.3	16.1	12.7	15.6	8.5	3.3	82	Broken clouds, hazy
May 9–11	17.9	23.6	25.6	24.3	20.1	8.0	13.1	13.9	13.7	16.1	9.6	3.0	77	Overcast, hazy
May 11–13	21.3	25.4	23.2	30.6	18.3	5.9	16.4	20.2	14.1	20.2	8.2	3.7	74	Light broken clouds, hazy
May 13–15	18.5	22.9	19.6	19.6	16.9	6.1	10.6	18.9	13.4	15.3	10.2	2.8	83	Broken clouds, hazy
May 15–17 (day and night temp. 26.5°C.)	15.1	21.7	24.2	27.0	27.5	27.1	16.4	20.8	23.6	25.8	26.3	18.6	60	Broken clouds
May 17–19 (day and night temp. 26.5°C.)	20.3	21.0	24.5	27.1	30.6	32.5	24.1	22.1	32.7	34.0	38.1	36.9	73	

experiments (table 3 in Went, 1944a). The stem growth rates of the other groups over the same 14-day period come remarkably close to the growth rates as expected from the curves in figure 4 from Went (1944a) as seen in table 2 with the exception of the 22° values, which were too low due to the incubator. This proves again the reproducibility of results obtained in the air-conditioned greenhouses, and it brands the experiment of tables 1 and 2 as thoroughly typical.

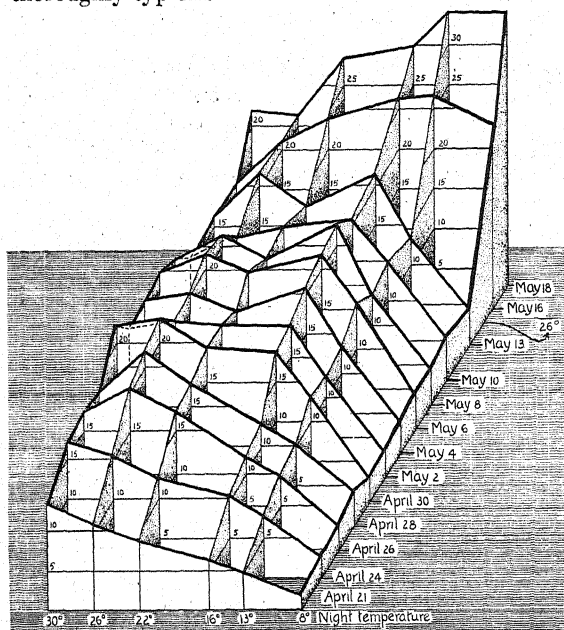


Fig. 1. At the various night temperatures (axis towards left) the growth rate in mm./day (vertical axis) is plotted for the San Jose Canner tomato. Growth rates are averaged for 26.5° and 18° day temperatures. Axis toward right shows age of tomato plants. On April 19 average length of plants was 10 cm. From May 15 on, all tomatoes were subjected to 26° night temperature. Values for 22° night temperature are seemingly too low as the plants grow taller. Same experiment as tables 1 and 2.

Figure 1 shows clearly the shift in optimal night temperature. Until April 25 the optimum lay at 30°C., which is probably very close to the optimal temperature of the growth process (cf. White, 1937). After the 25th the optimum shifted to 26°, and remained there until about May 3, when it shifted to 16°C. The growth rate of the plants kept at 22° during the night was usually slightly lower than the mean of 26° and 16°, and this is probably due to the fact that during the night they were enclosed in a 2 × 5 × 6 foot incubator, in which there was much less renewal of air than in the air-conditioned darkrooms in which the other plants were kept at night. This conclusion is strengthened by the observation that in a smaller, better insulated incubator (1.5 × 3 × 3 foot) the growth rate at 22° was still lower than in the larger incubator. But irrespective of the slight aberration of the 22° values,

TABLE 2. Final weight and length of tomato plants (San Jose Canner) harvested on May 19, 1944, each figure the mean of 5 plants, grown in one-gallon crocks in gravel (same experiment as table 1).

Day temperature	26.5°C.					18°C.					Mean of both groups				
Night temperature in °C.	30	26.5	22	16	13	8	30	26.5	22	16	13	8	30	26.5	22
Fresh weight in roots in grams.....	43	58	54	63	71	57	50	47	58	65	72	50	47	53	56
Fresh weight of tops in grams.....	182	201	168	183	170	91	167	148	147	133	112	65	175	175	168
Total stem elongation in mm. April 19-May 15	505	603	525	555	410	109	400	348	337	312	200	68	453	476	431
Stem elongation rate in mm./day. May 1-15....	20.4	24.7	23.5	26.4	18.8	5.9	15.1	17.4	13.8	15.8	9.3	3.2	17.8	21.0	18.7
Growth rate as extrapolated from figure 4 (Went, 1944a).....	19.7	23.1	26.5	26.5	18.7	5.5	...	18.0	...	15.8
Largest leaf, fresh weight in grams.....	10.5	11.8	13.1	10.8	10.5	7.8	8.4	8.9	9.7	13.1	9.3	4.8	9.5	10.4	11.4
Largest leaf, length in mm.....	356	338	324	308	277	212	280	308	306	296	260	190	318	323	315
Number of nodes on stem.....	23.4	23.2	21.8	22.0	21.7	18.4	22.6	19.2	21.0	17.8	18.2	17.2	23.0	21.2	21.4
Number of nodes between inflorescences.....	6.6	6.2	7.0	5.8	5.7	6.3	6.6	8.0	7.2	5.4	6.0	5.0	6.6	7.1	7.1
Relative development of inflorescence.....	2.0	2.4	2.6	3.2	4.0	3.0	1.4	2.6	3.0	2.2	2.2	2.8	1.7	2.5	2.8
Number of fruits set per plant.....	0	0	0	0	1.4	0.2	0	0	1.0	0.4	1.0	0	0	0	0.5
Ratio top/root fresh weight.....	4.2	3.5	3.1	2.9	2.4	1.6	3.4	3.2	2.6	2.1	1.6	1.3	3.72	3.30	2.82
Ratio of leaf (length) ² /fresh weight.....	116	102	78	89	75	57	107	93	93	69	73	75	108	98	84
Ratio of root weight/growth rate.....	2.1	2.3	2.3	2.4	3.8	9.7	9.3	2.7	4.2	4.1	7.7	15.6	2.6	2.5	3.0

it is very clear that the shift in optimum occurred very gradually. This can also be seen from the ratio of growth rates at 30° and 16° night temperature which gradually shifted from 1.96 to 0.83 (table 1) between April 19 and May 15.

On May 3, when the shift from 26° to 16° occurred, the plants had a mean length of 500 mm. which is about the length at which, in previous experiments, the shift from 26° to 18° optimal night temperature took place. It also can be seen that the shift was due to two factors: at 30° and 26° the growth rate, after reaching a maximal rate during the last days of April, gradually dropped to a lower level; at 8° and 13° night temperature there was a gradual and constant rise in growth rates, whereas, at 22° and 16° the maximal rate, once reached, was maintained.

After the measurements had been continued for 26 days, and the plants were nearing a size at which they could not be placed in the incubator at night, they were all subjected to the same conditions for the last four days (May 15–19), namely, a constant

day and night temperature of 26.5°C. Their response is shown in table 1 and figure 1.

Whereas the growth rate of the plants originally growing at 26.5° night temperature remained about constant, the growth rate of the plants previously kept at 30°C. during the night rose to about the same level as that of those kept at 26.5°, showing an immediate adjustment to the new growing conditions. The growth rate of the plants, kept previously at 8° and 13°C. during the night, increased rapidly to rates not even attained under optimal growing conditions, especially when the day temperature was raised as well. At intermediate temperatures intermediate responses were obtained. If the plants had been left longer at the same constant condition of 26.5°C., their growth rates would all have reverted to the steady rate of 23 mm. day (cf. curve F in fig. 5, Went, 1944a).

In table 2 a number of data concerning these same tomato plants at the time of harvest are recorded. For top weight the higher day and night temperatures were optimal, but the roots were heaviest after cool nights. The ratio top/root weight steadily de-

TABLE 3. *Fresh weights (in grams), number of fruits setting and growth rates in mm./day, all per plant for 5 different tomato varieties (see figure 3).*

Variety	Day temperature		26.5°C.					17°C.				
	Night temperature	30°	26°	22°	16°	13°	8°	26°	22°	16°	13°	8°
Earliana												
Root weight		30	57	53	53	29	28	53	(35)	45	57	28
Top weight		174	206	204	170	115	47	155	(178)	163	128	42
Fruit weight		10	1	118	67	36	6	2	(120)	108	21	1
Fruits per plant		1.7	0.7	8.7	6.3	3.7	1.0	1.0	(7.0)	6.3	3.3	0.7
Growth rate 26V–8VI.....		19.4	23.3	21.1	16.6	16.2	3.4	11.9	(12.0)	10.5	9.6	2.1
Growth rate 8VI–22VI....		24.0	31.9	26.2	25.6	25.7	5.9	18.4	(18.0)	16.1	14.6	3.3
Marglobe												
Root weight		29	32	72	63	40	37	dead	...	74	54	30
Top weight		123	196	260	244	139	76	dead	...	166	138	50
Fruit weight		0	0	78	123	14	0	dead	...	40	0	0
Fruits per plant		0	0	4.0	7.5	5.0	0	dead	...	3.0	0	0
Growth rate 26V–8VI.....		16.9	24.3	24.2	22.7	21.5	3.7	dead	...	9.3	7.4	1.5
Growth rate 8–22VI.....		7.8	13.9	20.1	23.1	25.5	5.9	dead	...	13.5	6.4	3.4
Pearson												
Root weight		23	49	65	72	52	29	61	...	57	46	21
Top weight		132	206	209	209	156	52	145	...	175	108	29
Fruit weight		0	0	22	55	11	0	0	...	6	0	0.3
Fruits per plant		0	0	4.7	4.7	1.7	0	0	...	0.7	0	0.2
Growth rate 26V–8VI.....		14.9	19.6	19.6	17.4	15.7	3.1	9.4	...	9.5	5.6	1.7
Growth rate 8–22VI.....		18.1	26.2	23.5	23.2	23.8	4.5	15.0	...	15.7	12.6	2.7
Stone												
Root weight		40	72	84	89	87	40	79	(62)	85	86	27
Top weight		172	197	243	209	180	65	189	(177)	184	110	32
Fruit weight		0	0	31	41	13	0	0	(3)	5	2	0
Fruits per plant.....		0	0	3.7	4.7	2.7	0	0	(1.0)	2.0	0.5	0
Growth rate 26V–8VI.....		17.3	17.6	18.8	17.3	14.4	2.9	12.0	(9.0)	7.8	6.4	1.3
Growth rate 8–22VI.....		17.1	30.3	23.0	21.8	23.2	5.3	14.0	(16.5)	16.8	10.0	2.6
Beefsteak												
Root weight		55	62	69	74	62	34	112	...	71	67	19
Top weight		234	254	240	202	178	61	215	...	188	136	33
Fruit weight		0	0	52	44	9	0	0	...	1	5	1
Fruits per plant		0	0	5.0	4.0	1.0	0	0	...	0.2	0.5	0.2
Growth rate 26V–8VI.....		22.4	24.8	21.2	17.6	15.8	3.3	13.5	...	10.0	6.6	1.9
Growth rate 8–22VI.....		23.7	29.5	25.1	27.6	27.5	5.7	17.1	...	18.8	10.6	3.7

creased with decreasing night temperature and was also consistently lower at the lower day temperature. A similar effect of temperature on root development was observed by Cochran (1936) for peppers. Leaves were slightly larger at the higher night temperatures, but the weight was highest at medium night temperatures. By dividing the leaf surface (proportional to the square of the leaf length, since

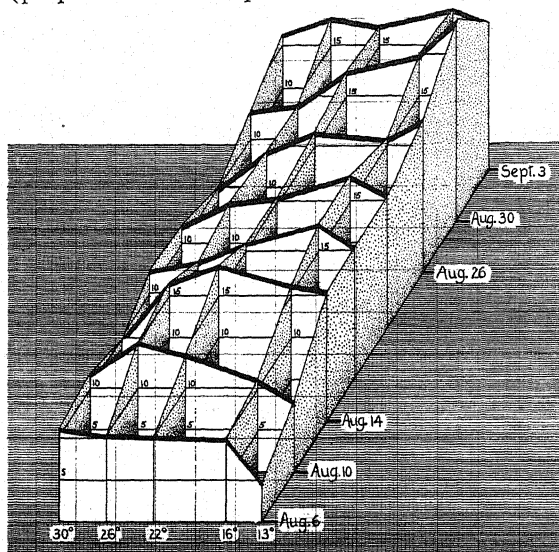


Fig. 2. Growth rates of the Illinois T19 tomato (vertical axis) in mm./day, kept during day at 26.5°, and during night at different temperatures (axis towards left). The same plants have been measured at about 4-day intervals (axis towards right).

the leaf proportions are not much affected by temperature) by the leaf weight, a value is obtained which gradually decreased with decreasing night temperature, indicating that the leaves were thicker in plants kept cool at night.

The effect of night temperature on total number of stem nodes, or on the number of stem nodes between inflorescences, was rather slight, which is in agreement with earlier observations on the tomato plant (Went, 1944b); but in this respect the tomato differs from many California annual plants (Lewis and Went, 1945), in which the rate of meristematic differentiation in the stem tip was correlated with the night temperature.

The size of the inflorescence varied very much with night temperature; the warmer the night, the smaller the inflorescence and the flowers. In table 2 the inflorescences were graded as follows: (1) very small and abortive, (2) small, (3) fair-sized, (4) large. Fruit set went parallel with flower development. Only at 22° and 13° night temperature did fruits start to grow; at higher temperatures no fruit was set at all.

Previously it had been shown (Went, 1944c) that although the optimal temperature of the growth process lies around 30°C., the optimal night tem-

perature (when most of the stem elongation occurs) for the tomato plant as a whole is lower, due to a decrease in sugar translocation at higher temperatures. This leads to various conclusions: (a) As the plant grows taller, translocation becomes more and more limiting, and consequently the optimal temperature for stem elongation shifts to a lower level. (b) When translocation is limiting, the roots should be most affected since they are farthest removed from the leaves. Actually the roots became proportionally lighter the higher the night (and day) temperature. (c) When translocation of sugars is limiting above 18°C., the growth process uses all sugars as soon as they arrive, and no storage of sugar occurs. Tomato plants which had been growing at night temperatures above 18°C., therefore, immediately assumed the growth rate typical of the new temperature after transfer to 26°C. night temperature. On the other hand, when they were grown at night temperatures below 18°, not all sugar transferred to their growing stems had been used, some

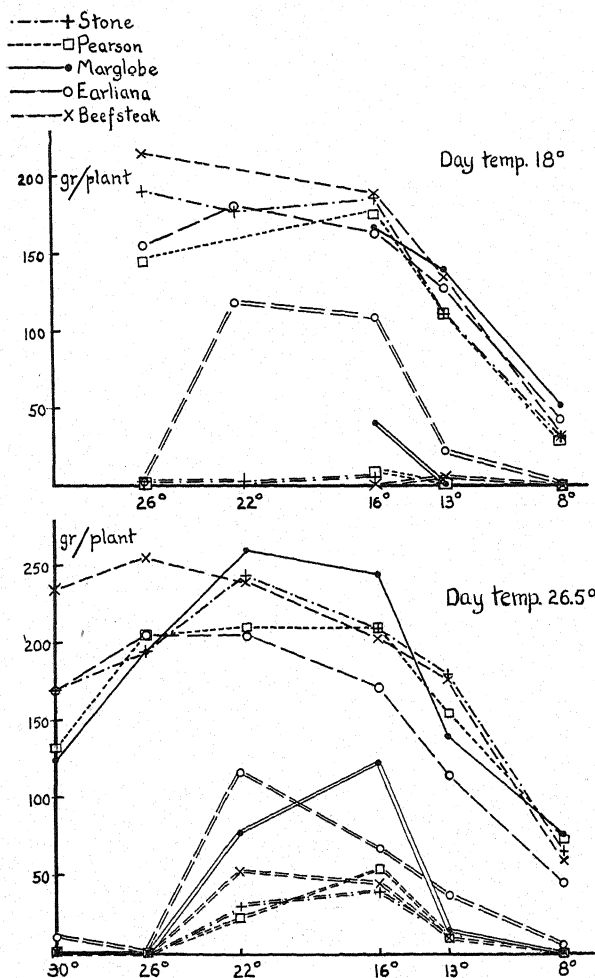


Fig. 3. Top weight (single lines) and fruit weight (double lines) of the tomatoes shown in table 3. On ordinate is shown weight in grams fresh weight per plant, on abscissa night temperatures, in degree centigrade.

TABLE 4. *Fresh weight in grams per plant and growth rates in mm./day for 10 different tomato varieties grown simultaneously in the air-conditioned greenhouses (see figure 5).*

Variety	Day temperature		26.5°C.						17°C.				
	Photoperiod hours		8	8	11	8	8	8	8	8	8	11	
	Night temperature	30°	26°	26°	22°	16°	13°	26°	22°	16°	13°	13°	
Essex Wonder													
Root weight		20	20	35	33	32	21	...	38	31	29	47	
Top weight		140	178	271	202	251	142	...	241	213	150	168	
Fruit weight		0	0	2	11	40	3	...	1	0	0	1	
Growth rate 14-29X.....		23.4	27.3	28.9	26.0	32.2	20.8	...	18.2	13.6	14.2	12.9	
Growth rate 29X-14XI....		22.8	28.0	35.6	28.7	32.6	26.0	...	21.4	19.4	17.5	13.5	
Comet Forcing													
Root weight	12	21	14	16	20	14	...	23	23	...	
Top weight	121	233	170	238	108	110	...	158	135	...	
Fruit weight	0	1	13	43	9	1	...	5	0	...	
Growth rate 14-29X.....		...	21.6	25.3	24.9	28.6	21.9	13.7	...	13.5	11.4	...	
Growth rate 29X-14XI....		...	20.2	31.9	28.2	32.6	23.5	15.8	...	15.4	12.6	...	
Valiant													
Root weight		11	12	40	...	27	20	16	29	22	28	31	
Top weight		81	121	277	...	211	111	94	176	126	117	115	
Fruit weight		0	0	6	...	4	6	0	1	0	0	1	
Growth rate 14-29X.....		17.7	22.4	26.7	...	26.8	20.8	13.2	13.2	12.7	12.1	11.1	
Growth rate 29X-14XI....		20.8	25.6	30.0	...	28.2	22.4	13.7	14.9	13.5	11.9	13.8	
Michigan State													
Root weight	18	42	...	24	30	...	40	30	36	...	
Top weight	170	276	...	176	187	...	254	166	166	...	
Fruit weight	0	0	...	1	3	...	3	0	0	...	
Growth rate 14-29X.....		...	23.2	25.2	...	23.6	21.2	...	13.1	12.4	10.4	...	
Growth rate 29X-14XI....		...	30.0	31.9	...	26.3	23.5	...	17.7	14.6	10.1	...	
Santa Clara Canner													
Root weight		12	13	31	19	30	23	10	...	22	...	45	
Top weight		86	108	227	124	186	121	63	...	126	...	207	
Fruit weight		0	0	0	0	0	0	0	...	0	...	0	
Growth rate 14-29X.....		14.0	19.6	24.9	22.0	23.6	22.2	9.8	...	12.7	...	9.8	
Growth rate 29X-14XI....		17.0	23.9	30.8	23.3	29.5	20.1	9.1	...	14.1	...	11.4	
Norton Stone													
Root weight	14	42	...	32	30	13	...	26	...	54	
Top weight	127	228	...	223	132	74	...	120	...	175	
Fruit weight	0	0	...	0	0	0	...	0	...	0	
Growth rate 14-29X.....		...	19.6	22.8	...	22.9	20.7	11.4	...	12.6	...	11.2	
Growth rate 29X-14XI....		...	22.3	25.5	...	23.8	24.5	12.1	...	15.7	...	11.5	
Marglobe													
Root weight		13	14	33	...	23	20	25	...	28	...	31	
Top weight		102	119	227	...	162	10	109	...	154	...	160	
Fruit weight		0	0	1	...	0	0	0	...	0	...	0	
Growth rate 14-29X.....		15.8	19.4	18.4	...	22.4	15.4	9.8	...	11.4	...	11.7	
Growth rate 29X-14XI....		20.4	25.6	26.0	...	28.7	17.9	15.6	...	14.8	...	11.0	
Rutgers													
Root weight		15	16	26	21	21	24	14	28	21	17	...	
Top weight		111	146	189	149	208	122	83	165	117	76	...	
Fruit weight		0	0	16	0	1	0	0	0	0	0	...	
Growth rate 14-29X.....		12.3	18.3	18.6	18.8	18.3	17.4	11.1	8.4	9.8	9.0	...	
Growth rate 29X-14XI....		17.6	21.6	21.3	22.2	26.2	20.2	10.6	14.2	14.3	8.5	...	
Indiana Baltimore													
Root weight		6	13	28	21	25	19	9	...	22	22	27	
Top weight		46	109	196	118	181	83	57	...	125	89	109	
Fruit weight		0	0	0	0	0	0	0	...	0	0	0	
Growth rate 14-29X.....		15.0	18.7	19.4	19.8	20.0	14.9	8.9	...	10.6	6.8	11.0	
Growth rate 29X-14XI....		13.3	23.5	26.5	20.6	24.4	16.1	9.1	...	15.3	10.9	11.8	
Burpee Jubilee													
Root weight		12	8	22	...	17	...	10	...	16	...	31	
Top weight		98	81	181	...	141	...	75	...	82	...	141	
Fruit weight		0	0	0	...	0	...	0	...	0	...	0	
Growth rate 14-29X.....		12.0	15.2	16.9	...	21.1	...	9.3	...	7.7	...	7.7	
Growth rate 29X-14XI....		15.2	19.3	24.1	...	22.9	...	10.8	...	8.6	...	9.1	

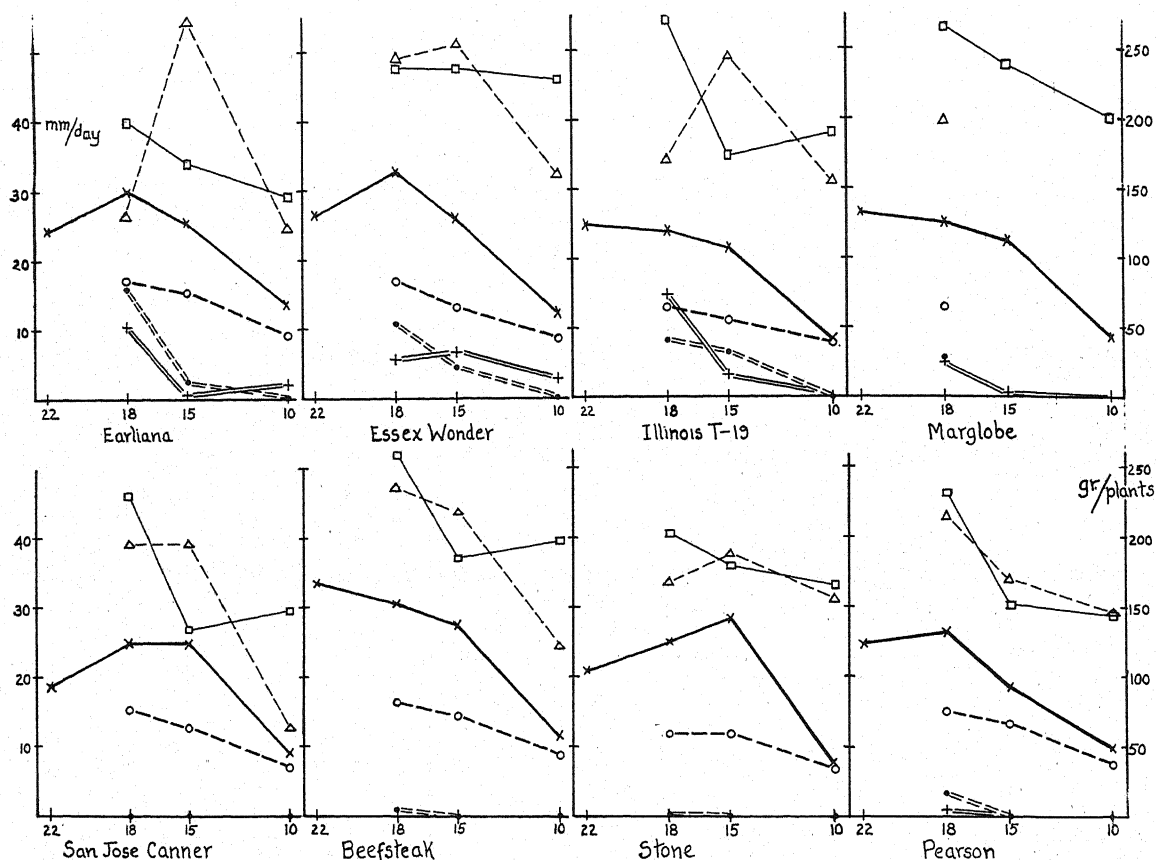


Fig. 4. Growth rates over a 20-day period of 8 different tomato varieties, in mm./day. Crosses and solid lines, day temperature 26°C.; circles and broken lines, day temperature of 18°C. Fruit weight in grams fresh weight per plant are shown for 26° day temperature (plusses and double solid lines) and for 18° day temperature (dots and double broken lines). Fresh weights of plants (squares and solid lines, 26° day temperature; triangles and broken lines, 18° day temperature) compared with those of table 3.

accumulation occurred, and after transfer to higher night temperatures the growth rate increased well beyond what was possible under steady conditions.

The present experiment, therefore, is in complete agreement with the assumed interaction of growth factors, and furnishes strong support for the conclusion reached in an earlier paper (Went, 1944c) that above 18° night temperature translocation of sugar limits the growth of a tomato plant as a whole, including stem, root and fruit growth.

RELATION BETWEEN VARIETY AND STEM GROWTH RATE UNDER VARIOUS TEMPERATURE TREATMENTS.—Similar experiments were carried out with other tomato varieties, and one such, in which the Illinois T19 was used, is shown in figure 2. The shift in optimal temperature was even more pronounced in this variety than in the San Jose Canner (fig. 1), and the optimal night temperature dropped as low as 13°C.

In tables 3 and 4 and figures 3, 4 and 5 the performance of 14 different varieties under various temperature treatments is compared. The size of the greenhouses and of the incubators made it impossible to continue the experiments beyond the

early stages of fruit setting, but some interesting varietal differences were evident. The general response of all varieties was approximately the same; faster growth and greater weight were obtained at the higher day temperature. The ratio between top and root weight decreased steadily with decreasing night temperature. Optimal fruit set for all varieties was at 16° or 22°C. night temperature, irrespective of day temperature. In the next paper of this series (Went and Cosper, 1945) the growth of tomato plants in the field will be compared with these greenhouse experiments.

In figure 4 some results of another experiment are shown. The late tomatoes (Beefsteak, Stone, Santa Clara Canner) had not started to set fruit when harvested, whereas Earliana, Essex Wonder and Illinois T19 had fruits setting on one or more flower clusters. As in the other experiments there was no correlation between growth rate and fruit set. The fastest growers (Beefsteak and Essex Wonder) belong to the late and to the early varieties. Although the growth rate at 26° day temperature was considerably higher than that at 18°, there was no consistent difference in fruit development at these two

day temperatures. Fruit set was often more extensive at the lower temperature.

Greenhouse tomatoes (Essex Wonder, Comet Forcing, Valiant and Michigan State) had the lowest optimal temperatures for growth and fruit set; they set earliest and at medium and low night temperatures had the highest growth rates (fig. 5). At the other end of the scale were the Eastern varieties (Rutgers, Indiana Baltimore, Burpee Jubilee) which had the lowest growth rates. Western and general purpose tomatoes (Santa Clara Canner, Norton Stone and Marglobe) were found to be intermediate between greenhouse and Eastern varieties in most respects, although considerable individual variations were found between varieties. For instance, the difference between Essex Wonder and Comet Forcing is interesting. Although the top and fruit weight of these two was about equal at 16° night temperature (which is optimal), the Essex Wonder apparently had a wider tolerance for higher and lower night temperature (c.f. top weight), and its root system was about twice as heavy as that of the Comet Forcing under similar conditions.

RELATION BETWEEN LIGHT INTENSITY AND OPTIMAL NIGHT TEMPERATURE.—In previous papers (Went, 1944a) it was pointed out that light intensity had only a minor effect on stem elongation in the tomato; on overcast days their growth rate was slightly higher, but this increase was usually balanced by a subsequent decrease on the following sunny days. Table 1 does not show any pronounced correlation between cloudiness and growth rate; there is only a faint indication that partial cloudiness increased, and that a completely clouded sky decreased, the growth rate. A much clearer correlation between cloudiness and rate of stem elongation was found in tomato plants in 4-inch pots, which were placed between those in 1-gallon crocks, and were shaded by these larger plants. Whereas their general behavior was the same (gradual shift of optimal night temperature from 30° to 16°C.), their growth was subjected to much larger fluctuations,

and on cloudy days their growth rate at the higher night temperature was very low. Figure 6 shows this effect. The growth rates for the periods with completely overcast weather (April 27–29, May 1–3 and May 9–11) are compared with those on the partially cloudy or completely clear days (April 29–May 1, May 3–9 and May 11–15). Whereas cloudiness did not affect the growth rate at lower night temperatures, at 26° and 30° night temperature the rate of stem elongation, both in the 26° and 18° day temperature, was much lower on cloudy days. This resulted in a pronounced shift in the optimum towards lower night temperatures. It is evident, however, that this phenomenon occurred only when the light intensity became very low, since the tomatoes in 1-gallon crocks did not show a marked shift towards lower optimal night temperatures on cloudy days. It is likely to become of practical importance only during dark winter days in greenhouses at higher latitudes.

To check whether the shift to lower optimal night temperature was a direct response to a lower light intensity, a number of experiments were carried out under artificial light with the San Jose Canner tomato.

In one set of experiments, groups of 10 plants were subjected to 400 foot-candles at 26.5°C. for 24, 16 or 8 hours daily. The dark hours were spent at 26°, 16° and 8°C. In the course of 2–4 weeks many of the plants subjected to continuous light showed a complete bleaching of large areas of the younger leaves. A number of plants kept at 26.5°C. during darkness aborted their growing points, or they were very slow to develop a lateral shoot in the axil of the leaf immediately below the inflorescence. Table 5 shows the values for two sets of plants grown at different times. In general there is good agreement between the growth rate in the two experiments; in the first experiment, which lasted longer, the growth rates were higher in the case of those plants which were kept during the daily dark period at 26.5°C. This was due to a certain amount

TABLE 5. Sugar content, growth rate and final weight of small tomato plants, grown for 3–6 weeks under artificial light of 400 foot candles, and kept during their daily dark period at different temperatures. All sugar determinations mean of 2–3 duplicates. Each value mean of 10 plants.

Experiment June 6–July 5, 1944									
Sugar content of leaves in % dry wt.							Experiment August 3–19, 1944		
Hours in 26.5°C. at 400 f.c.	Rest of day in darkness	At beginning of light period		At end of light period		Growth rate mm/day	Growth rate mm/day	Total fresh wt. g/plant	Longest leaf in mm
		Red. sugar	Sucrose	Red. sugar	Sucrose				
24 hours	2.45	0.50	2.38	0.42	12.4	11.9	29.6	163
16 hours	26°C.	2.03	0.76	2.03	0.85	10.3	7.9	19.8	170
16 hours	16°C.	2.12	0.54	2.12	2.77	9.0	9.0	26.9	188
16 hours	8°C.	1.80	1.30	1.85	1.69	8.1	5.5	21.2	165
8 hours	26°C.	2.15	0.59	2.11	2.05	5.9	3.4	12.5	161
8 hours	16°C.	1.75	0.29	1.87	0.12*	9.9	10.0	18.6	176
8 hours	8°C.	1.97	0.68	2.17	1.03	3.3	3.4	16.6	161

* Value obviously too low. Lack of material precluded another analysis.

Photoperiod

8 8 10 8 8 8 8 8 10 8 8 8

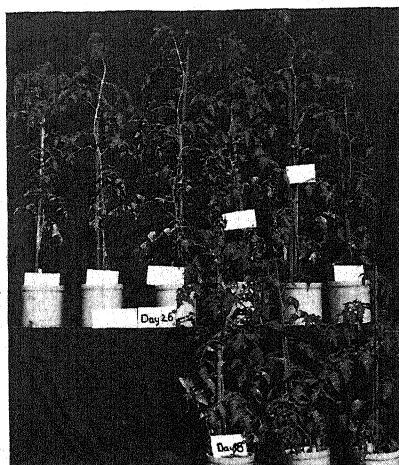
Night temp.

30 26 26 22 16 13 30 26 26 22 16 13

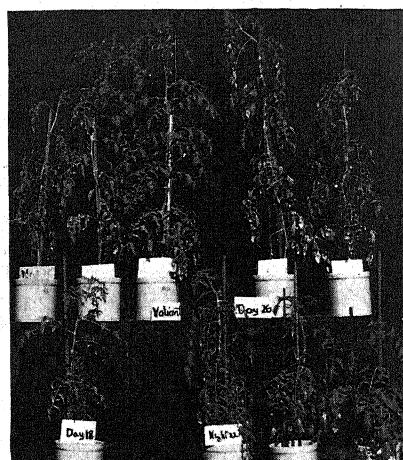
Day temp.

26.5°

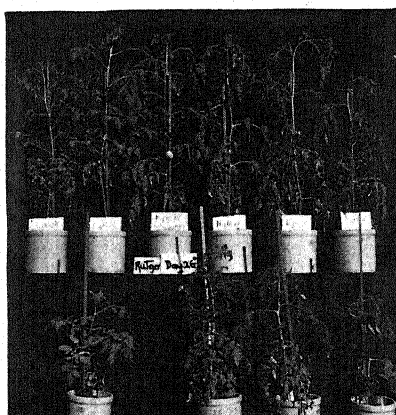
18°



Essex Wonder



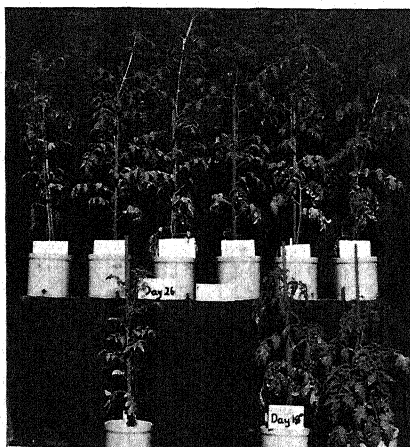
Valiant



Rutgers



Indiana Baltimore



Santa Clara Canner

 100
80
60
40
20
0
-20
-40
-60
-80
-100 cm.

 100
80
60
40
20
0
-20
-40
-60
-80
-100 cm.

 100
80
60
40
20
0
-20
-40
-60
-80
-100 cm.

Fig. 5. One typical plant from each temperature treatment referred to in table 4. Outside diameter of each crock at rim 20.5 cm. Upper row in each variety grown at day temperature of 26.5°, lower row at 18°. Upper two are greenhouse varieties, middle two are Eastern varieties, lower one is a typical California variety.

of "adaptation" since their growth rates were lower in the first than in the second half of the experiment. The fresh weight of the plants at the end of the experiment paralleled the differences in growth rate. For each length of photoperiod there was a negative correlation between sucrose content at the beginning of the light period and the growth rate. When illuminated for 8 hours only, the growth rate was highest at the dark temperature of 16° , which is almost optimal, whereas in a 16-hour photoperiod there was little difference in growth rate for 16° or 26.5° C. dark temperatures. Therefore, it can be concluded that whereas the optimal dark temperature is between 16° and 26.5° at a 16-hour photoperiod, the optimal dark temperature is near 16° for an 8-hour photoperiod at 400 foot-candle intensity.

In figure 7 the results of another experiment are shown. After an initial 12-day period tomato plants grown in 4-inch pots had become adjusted to the different light intensities and photoperiods, and figure 17 gives the growth data for the period 12–18 days after beginning of the light treatments. Plants were grown under the fluorescent lamps at various distances so that they received 400, 200 or 100 f.c. light. They were exposed for 8 or 16 hours daily to this light at 26.5° C. and the rest of the time they stayed at 26° , 16° or 8° C. in darkness. In the figure are plotted the means of the 16-hour photoperiod at 200 f.c. (3200 f.c. hr.) and of the 8-hour photoperiod at 400 f.c. (also 3200 f.c. hr.), and likewise those of the 16-hour photoperiod at 100 f.c. and 8-hour photoperiod at 200 f.c. (both 1600 f.c. hr.). The plants receiving 800 f.c. hours had stopped growing and died before the experiment was finished.

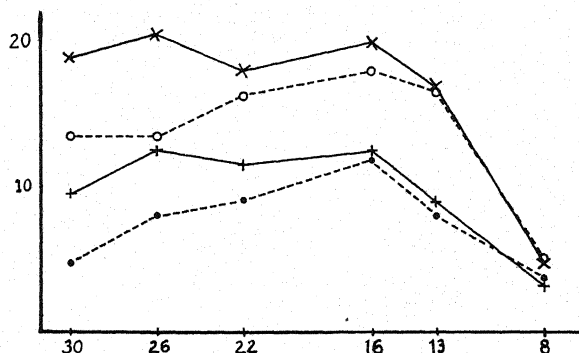


Fig. 6. Relation between growth rate of San Jose Canner tomato plants grown in 4-inch pots and night temperature. Upper two curves (crosses and circles) relate to plants grown during day at 26.5° C., lower two curves (pluses and dots) to those grown at 18° C. Solid lines: growth rate on clear days. Broken lines: growth rate on dark and cloudy days.

The plants receiving the most light (6400 f.c. hr.) had an optimal dark temperature of about 20° C. At their size (300 mm.) the optimal dark temperature would have been about 26° C. at the intensity of full daylight (as shown for the 13,000

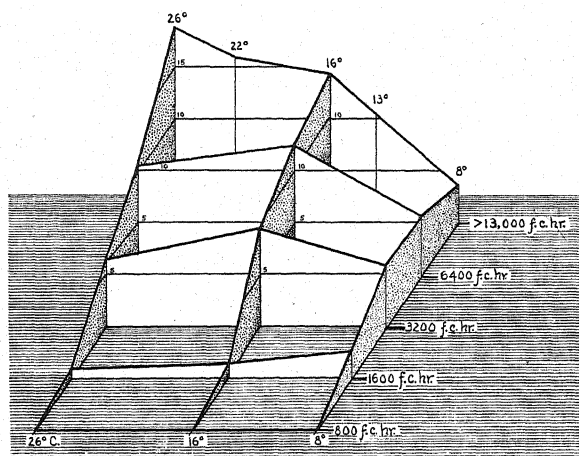


Fig. 7. Growth rates (vertical axis, mm./day) of young San Jose Canner tomato plants (about 20 cm. tall), daily receiving different amounts of light at 26.5° C. (axis towards right, foot-candle hours per day). During dark period various groups are kept at different temperatures (axis towards left).

f.c. hr.), which values were taken from table 1 for the April 22–25 period, when the tomatoes had approximately the same size. The group receiving 3200 f.c. hours had an optimal dark temperature very close to 16° C., but at the next lower amount of light the optimum lay near 8° C.

In the same experiment two different temperatures during the photoperiod were compared. Whereas in full daylight the optimal night temperature was 26.5° C., and 18° C. day temperature supported only 60 per cent as much growth; at 200 f.c. 18° C. day temperature gave consistently better growth than 26.5° C.

This shift towards lower optimal light and dark temperatures when tomato plants are exposed to lower light intensities or to less light has been known among greenhouse growers. Dr. H. O. Eversole (see Hiatt, 1941) found this to be true for *Phalaenopsis*; he always reduces the temperature in his greenhouse on cloudy days, and also lowers the temperature during the succeeding night. Under Ohio greenhouse conditions in spring the best day temperature for Globe tomatoes is 18° on cloudy and 21 – 24° C. on sunny days (Hoffman, 1938).

Only one investigation has been published in which the relation between light intensity and temperature has been investigated quantitatively (Brandes and Lauritsen, 1940). With a 14.5-hour photoperiod they grew 11 varieties of sugar cane at 15° and 25.5° C. and 33 foot-candles, and also at 25.5° C. and 90 f.c. In all cases the early growth was greater at the higher temperatures, but invariably the growth at high temperature and low light intensity stopped after 5–6 weeks, whereas at high temperature and medium light intensity growth continued, but at a decreasing rate; and at low temperature and low light intensity growth continued

at a constant though slow rate. This experiment with sugar cane shows the same response to light and (constant) temperature as exhibited by the tomato.

TEMPERATURE AND DISEASE INCIDENCE.—As a result of growing tomatoes without interruption in the greenhouse and in the adjoining field for a period of several years, virus diseases gradually became established in the plants, and many of the plants referred to in table 4 were diseased, which is the reason why the experiment was discontinued before the same stage of growth was reached as that shown in table 3. Some marked differences in virus symptoms were observed. When subjected to a day temperature of 18°C., shoestring virus was common, but at 26.5°C. day temperature it did not occur. No apparent connection with night temperature was observed. At the higher day temperature mosaic was slightly more prevalent, but the latter disease was strongly correlated with high night temperatures. At 13° night temperatures no mosaic symptoms were visible; at 16° they were hardly discernible, at 22° and 26° the diseased plants were very apparent, and at 30° night temperature the plants having mosaic showed systemic effects and were dwarfed. These differences persisted in spite of the fact that diseased and healthy plants were in close contact during day or during night. Spotted wilt developed only at high night temperatures.

DISCUSSION.—Repeatedly in the present paper the reproducibility of results has been stressed. This means not only that the law of causality governs the growth of tomato plants, but also that the most essential conditions for tomato growth were controlled, and the necessary qualifications for the condition of the plant were known. The multidimensional concept of causality has been helpful in this respect. This is most clearly exemplified by figures 1, 2 and 5. In figure 1 all values are stem elongation rates of San Jose Canner tomatoes, grown under identical daytime conditions in an 8-hour photoperiod in full daylight (the data for 26° and 18° day temperatures were combined). For plants grown at 26.5°C. night temperature the growth rates ranged from 10–34 mm. per day. The steady growth rate at this night temperature for a full-grown plant was about 21 mm./day. When the plants were small, rates as low as 10 mm. were found, rising to a maximum of 23 mm. But the growth rates of 21–34 mm./day observed on May 18 were also found at a night temperature of 26.5°C. In the latter case the previous history of the tomatoes had to be taken into account. Such interrelationships between growth rate and night temperature on the one hand, and modifying factors on the other hand can be expressed in multidimensional graphs, which stress the complex causality existing between these factors.

Although biologists are used to the complexity of living material, they seldom have had the means of adequately expressing complex interrelation-

ships, since environment could not be controlled sufficiently.

Curves similar to figures 1 and 2 were prepared for a number of other varieties (those shown in tables 3 and 4), and thus another variable was introduced, namely, varietal response, thus requiring four dimensions to portray growth in conjunction with age and night temperature. This can be accomplished by having a series of three-dimensional models lined up in a row.

As figure 7 shows, a portrayal of the relationship between total daily radiation and night temperature as it affects stem elongation in young plants is also three-dimensional. When combined with the dimensions of time (age of plants) and variety, a five-dimensional presentation becomes necessary. Similar five-dimensional pictures could portray fruit production or root development.

Additional dimensions could be added by considering day temperature, photoperiod and nutrition. It may be argued that the picture then becomes altogether too complicated. On the other hand, unless we know all interrelations between environmental factors and the organism, no clear-cut picture is possible and we will remain in the indefinite stage we are now in, describing experiments as far as possible but not expecting complete reproducibility because the response may be different under other conditions. A multidimensional presentation, on the other hand, shows exactly the expected response under any combination of conditions.

To what extent the data collected in the present experiment are reproducible is seen by consulting the growth rates in table 2, lines 4 and 5. There are other conditions, however, which are not sufficiently controlled to give reproducible results. In table 8 of Went (1944a), for instance, it can be seen that during June and July a variation of the photoperiod between 8 and 14 hours had no appreciable effect on the dry weight of San Jose Canner tomatoes. In table 4 of the present paper, however, the data show that subjection of plants to full daylight during October and November almost doubled their wet weight at 26.5°C. day and night temperatures, compared with an 8-hour photoperiod. At 18° day and 13° night temperature no difference was found due to length of photoperiod. This was true for the San Jose Canner also (not shown in table 4). The length of photoperiod, therefore, affected total weight more than stem elongation, and was more effective during the darker autumn days than during the lighter summer days.

The experiments presented above show that thermoperiodicity is not a phenomenon restricted to a single tomato variety, but it occurs in all 16 tomato varieties tested. There are quantitative differences between varieties; varieties which normally grow under similar conditions respond more uniformly than varieties adapted to different conditions. These differences in physiological response are consistent and large enough so that they can be used to characterize the varieties.

SUMMARY

The thermoperiodicity of tomato plants was studied in detail, considering interrelations between age of plant, light intensity and variety on the one hand, and stem elongation at six night and two day temperatures on the other hand.

It was found that a gradual shift of the optimal night temperature occurred, from 30°C. in small plants to 18°C. for the San Jose Canner and 13°C. for the Illinois T19 in the early fruiting stage.

A similar response was found in 14 other tomato varieties, but they each had slightly different temperature characteristics. In general the English and Greenhouse varieties grew fastest and had the lowest optimal night temperatures. Western varieties had the highest optimal night temperatures,

and Eastern varieties were intermediate between the other two as far as night temperature was concerned, but had the lowest absolute growth rates.

When the tomato plants were grown in full sunlight, their optimal night temperature was higher than on cloudy days, provided they were shaded by other plants. In artificial light the optimal night temperature fell off very rapidly with decreasing total illumination. Incidence of virus diseases was greatly modified by both day and night temperature.

In the discussion it is pointed out that these complex interrelationships are examples of a multidimensional causality, which can be presented properly only in multidimensional models.

WILLIAM G. KERCKHOFF LABORATORIES,
CALIFORNIA INSTITUTE OF TECHNOLOGY,
PASADENA, CALIFORNIA

LITERATURE CITED

- BRANDES, E. W., AND J. I. LAURITSEN. 1940. A required photothermal balance for survival and growth of sugar cane. *Sugar Bulletin*: 18:3-5.
- CÓCHIRAN, H. L. 1936. Some factors influencing growth and fruit-setting in the pepper (*Capsicum frutescens* L.). *Cornell Univ. Agric. Exper. Sta. Mem.* 190: 1-39.
- HIATT, G. 1941. Recent developments in the culture of *Phalaenopsis* in gravel. *Orchid Digest* 5:35-38.
- HOFFMAN, I. C. 1938. Present cultural methods in growing the spring greenhouse tomato crop in Ohio. *Ann. Rept. Veget. Growers Assn. Am.* 1938:88-100.
- LEWIS, H., AND F. W. WENT. 1945. Plant growth under controlled conditions IV. Response of California annuals to photoperiod and temperature. *Amer. Jour. Bot.* 32:1-12.
- WENT, F. W. 1944a. Plant growth under controlled conditions II. Thermoperiodicity in growth and fruiting of the tomato. *Amer. Jour. Bot.* 31:135-150.
- . 1944b. Morphological observations on the tomato plant. *Bull. Torrey Bot. Club* 71:77-92.
- . 1944c. Plant growth under controlled conditions III. Correlation between various physiological processes and growth in the tomato plant. *Amer. Jour. Bot.* 31:597-618.
- , AND LLOYD COSPER. 1945. Plant growth under controlled conditions VI. Comparison between field and air-conditioned greenhouse culture of tomatoes. *Amer. Jour. Bot.* In press.
- WHITE, P. R. 1937. Seasonal fluctuations in growth rates of excised tomato root tips. *Plant Physiol.* 12:183-190.

A MORPHOLOGICAL, DEVELOPMENTAL, AND CYTOLOGICAL STUDY OF FOUR SAPROPHYTIC CHYTRIDS. II. RHIZOPHYDIUM CORONUM HANSON¹

Anne Marie Hanson

RHIZOPHYDIUM CORONUM was found on bits of cellophane twelve days after they had been added to a handful of dry dust and distilled water. The dust was collected from a clay bank near Sharon, Connecticut. A great variety of substrata in various suspensory liquids was tried for culturing the fungus, but to date all have been inadequate. Observations recorded in this article were made chiefly from material present in the original sample which was practically unifungal. Some observations, however, were made on material from subsequent collections when *R. coronum* was predominant over other forms. In the latter instances, portions bearing only *R. coronum* were cut out, rinsed with distilled water, and maintained in the same liquid when not under observation. Reinfection does not take place in distilled water, but the thalli remain in good condition for several days.

The general type of development, structure, or-

ganization, and life cycle of *R. coronum* are so similar to those of other species of *Rhizophyidium* that it is unnecessary to describe them in detail. The present description will therefore be limited to cytological details, the development of the corona around the sporangia and other specific characteristics. As shown in figures 5 to 13, the germinating zoospore on the surface of the substratum enlarges to become the incipient sporangium, while the germ tube elongates and branches to form the rhizoidal system.

As soon as the latter is firmly established in the substratum, the gelatinous sporangial hull which characterizes the chytrid begins to develop. It first becomes visible as a hyaline envelope around the young incipient sporangium (fig. 8), and stains deep magenta with ruthenium red, indicating that the substance is pectinaceous. The hull is also stainable with janus green and neutral red, staining blue-green with the former and rusty red with the

¹ Received for publication June 13, 1945.

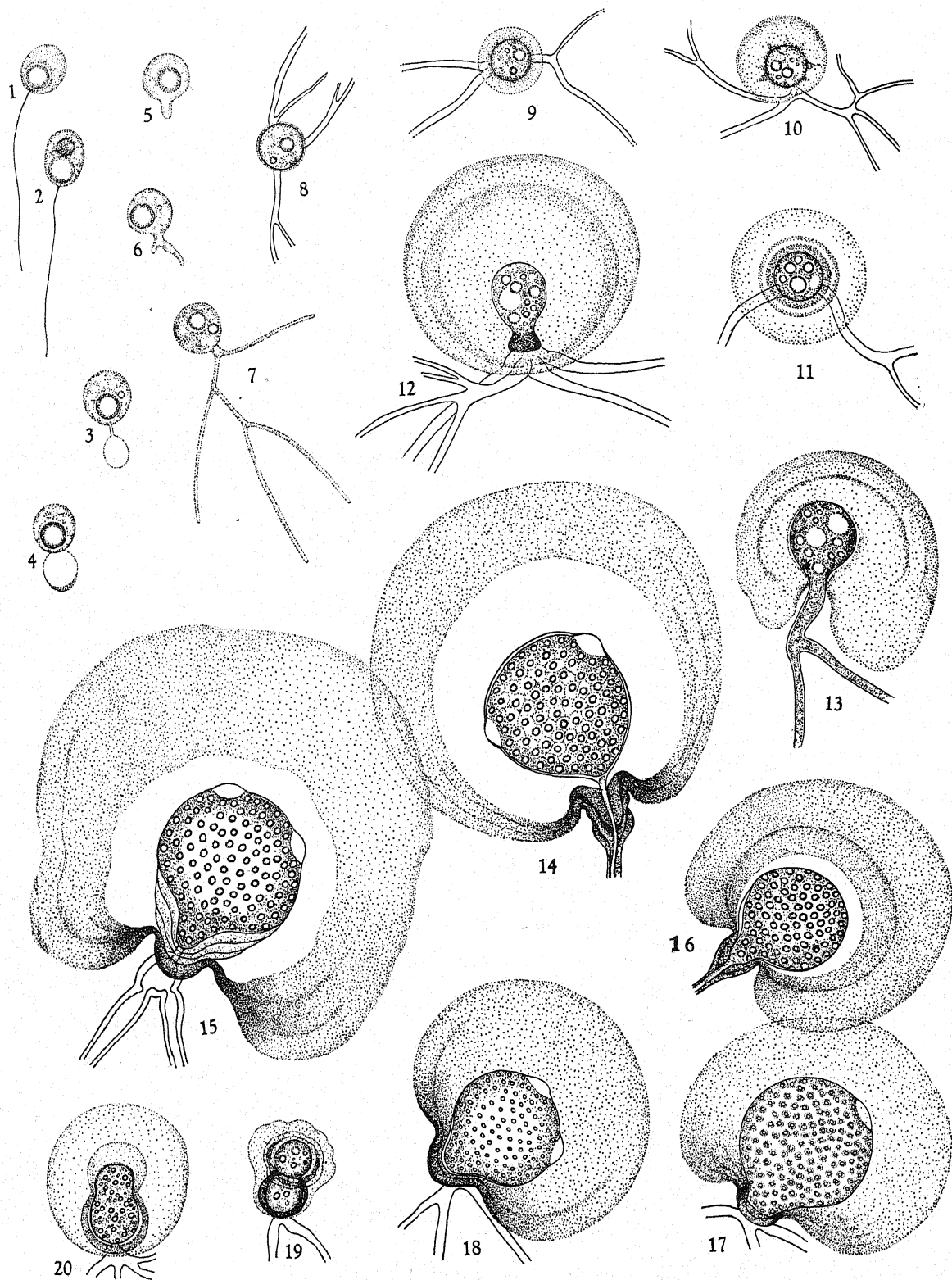


Fig. 1-20.

(See page 484 for complete legends for fig. 1-61.)

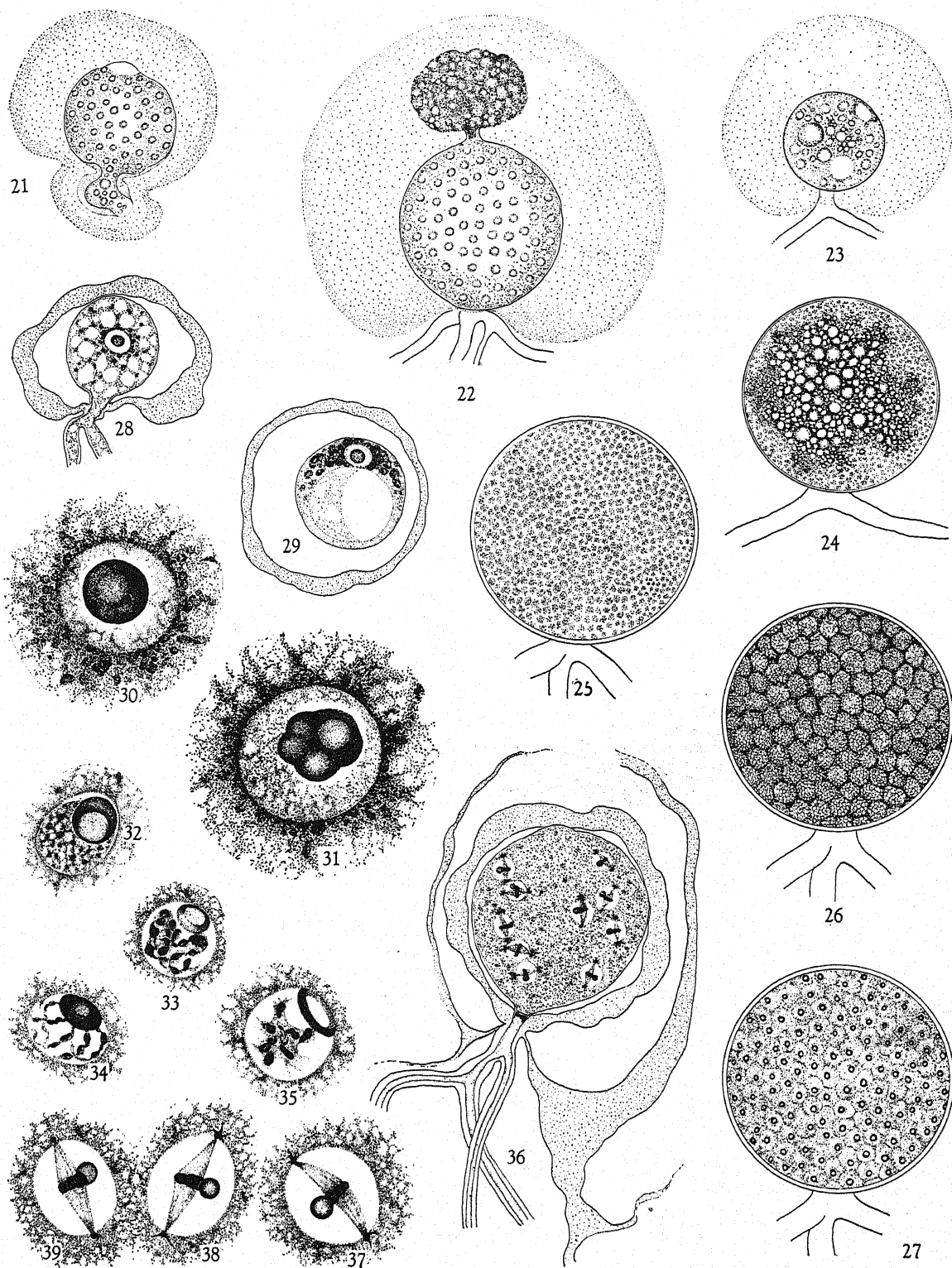


Fig. 21-39.

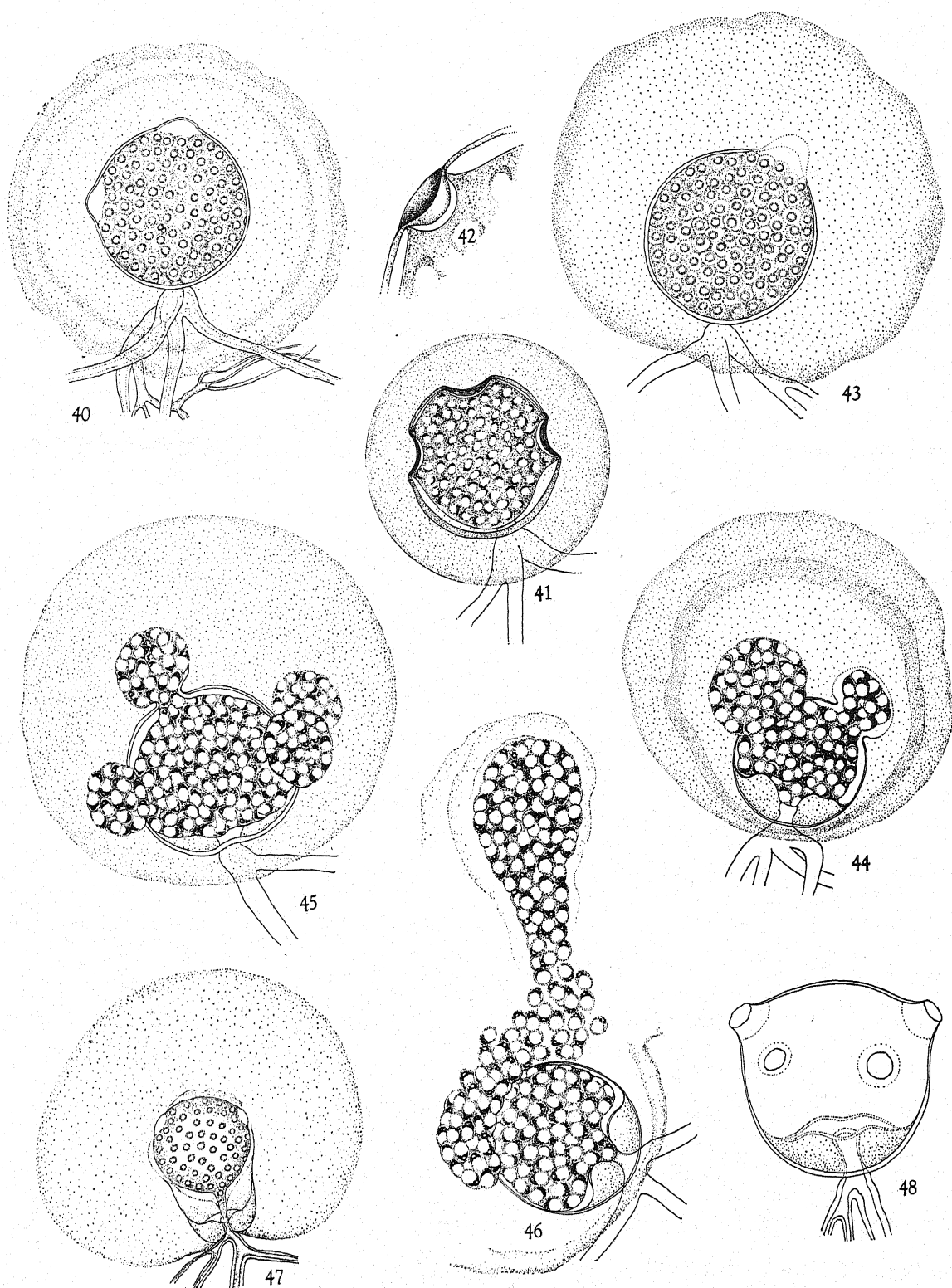


Fig. 40-47.

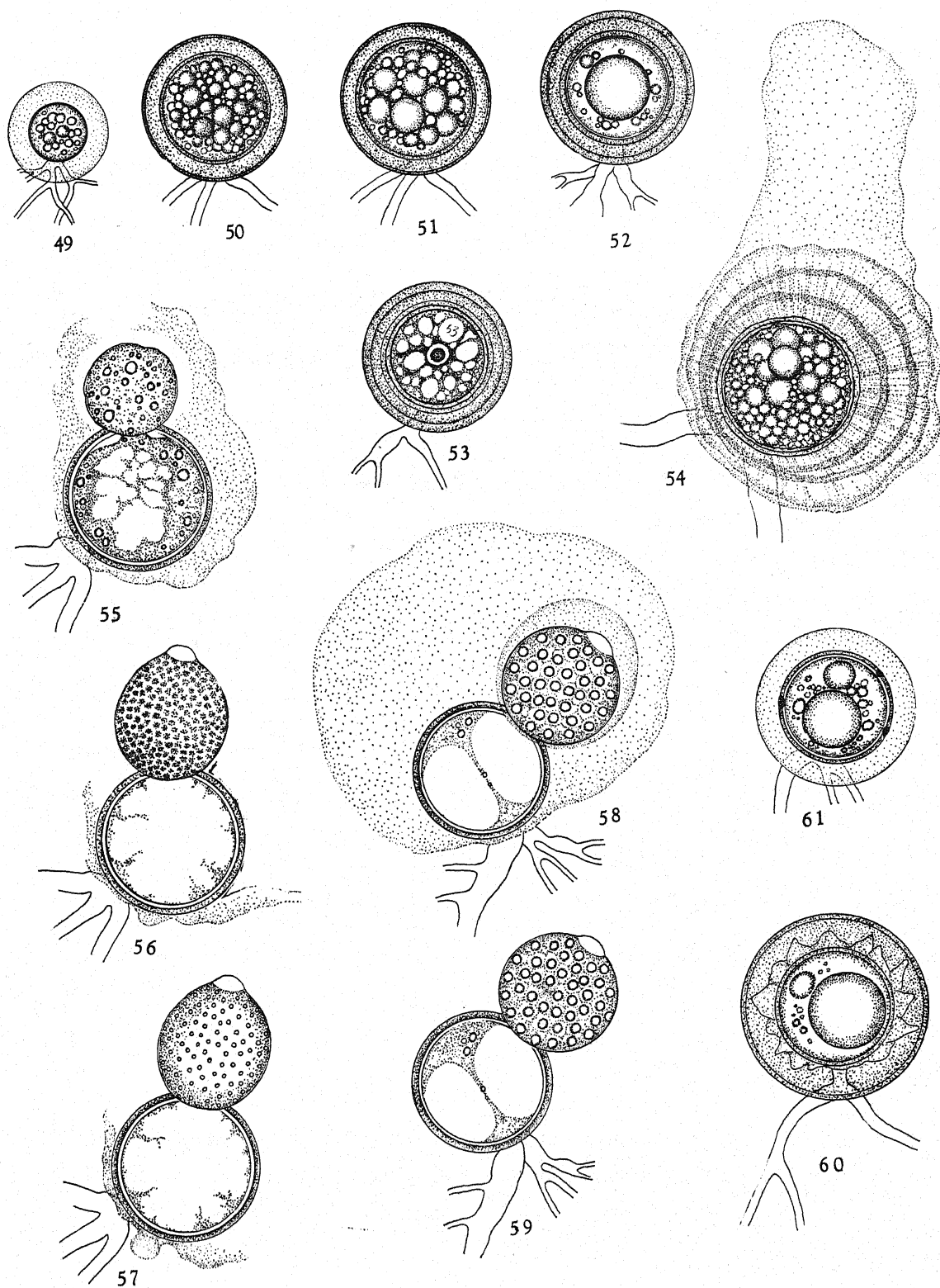


Fig. 49-61.

latter. In fixed preparations it is stainable with gentian violet and orange G. While the incipient sporangium is still quite small, the enveloping matrix gradually swells and becomes more transparent (fig. 9). The expanding hull, however, is not always optically homogeneous. Denser areas frequently radiate from the sporangial wall (fig. 10), and later two or more clear-cut concentric zones are often visible (fig. 11-13, 16). Ordinarily, the number of zones is two or three, but occasionally thalli with six zones in the hull may be found. The width and density of the zones are variable, and they converge toward the base of the sporangium (fig. 12, 14-18). The region of convergence of the zones surrounding the base of the sporangium may at maturity become deep golden to amber in color while the rest of the hull remains hyaline and transparent (fig. 14-18). The latter phenomenon is particularly prevalent in old cultures.

In addition to the normal type of development shown in figures 5 to 13, variations frequently occur in *R. coronum*. For instance, incipient sporangia may cease development and go into a dormant phase for several days. In the interim a portion of the hull immediately surrounding the sporangium may become very dense (fig. 19, 20). When growth is resumed, the sporangium does not enlarge uniformly, but chiefly at the apex (fig. 19, 20). In this manner elongate sporangia with median constrictions may often develop. In other instances when growth is renewed the content grows out of the original sporangium and forms a new and larger sporangium at the apex in a manner similar to rest-

ing spore germination, as is shown in figure 21. The original arrested sporangium in such cases persists as a basal appendage or apophysis. The hull of such sporangia is asymmetrical and roughly follows the outline of the initial sporangium and its outgrowth. Sporangial outgrowths do not always enlarge to form new and larger sporangia, but may encyst and become dark brown (fig. 22). When this takes place, the encysted outgrowth remains attached all during the development of the initial sporangium, but at the time of spore discharge it often breaks off.

The exact source of its material and manner of formation of the corona are not clearly evident. Its growth and expansion suggest the possibility that it may be an exudate formed by the sporangial protoplasm which passes through the wall and adheres to the surface as a hull. On the other hand, it may possibly result from gelatinization of the sporangial wall. No marked diminution in thickness of the wall has been observed during the origin of the corona. This in itself, however, is not significant, for if the outer wall material gelatinizes as suggested, new wall material may be deposited on its inner surface. In this event, the wall would maintain the same thickness. If the corona originates by gelatinization of the sporangial wall as suggested above, the zones are interpretable as resulting from progressive gelatinization and swelling of concentric lamellae composing the sporangial wall. On the other hand, the zones may also be interpreted as intermittent exudations from the sporangial protoplasm.

While the incipient sporangium enlarges and its

Fig. 1-20.—Fig. 1, 2. Actively swimming zoospores. $\times 3360$.—Fig. 3, 4. Zoospores with flagellar loops. $\times 3360$.—Fig. 5-7. Stages in germination of zoospores. $\times 3360$.—Fig. 8-10. Early stages in the development of the gelatinous hull of the incipient sporangium. $\times 3360$.—Fig. 11. Apical view of a zonated hull. $\times 3360$.—Fig. 12. Lateral view of a zonated hull. $\times 3360$.—Fig. 13. Incipient sporangium with hull in tangential section. $\times 3360$.—Fig. 14-18. Coronal modification at the base of the sporangium. $\times 840$.—Fig. 19, 20. Resumption of growth in young dormant sporangia. $\times 840$.—

Fig. 21-39.—Fig. 21. Sporangium with outgrowth developing into a large sporangium while the initial sporangium remains attached like an apophysis. $\times 840$.—Fig. 22. Sporangium with apically encysted outgrowth. $\times 1680$.—Fig. 23, 24. Accumulation of refractive material in the sporangial protoplasm. $\times 1680$.—Fig. 25. Evenly granular stage. $\times 1680$.—Fig. 26. Aggregation of granules in groups. $\times 1680$.—Fig. 27. Cleavage of sporangial contents into polyhedral zoospore initials. $\times 1680$.—Fig. 28. Uninucleate incipient sporangium in section. $\times 1680$.—Fig. 29. Young stage of incipient sporangium. $\times 5040$.—Fig. 30. Primary nucleus. $\times 5040$.—Fig. 31. Primary nucleus in early prophase with vacuolate nucleole. $\times 5040$.—Fig. 32. Prophase reticulum oriented on nucleolus. $\times 5040$.—Fig. 33. Later prophase with nodular chromosomes. $\times 5040$.—Fig. 34. Elongate nodular chromosomes oriented on heterogeneous disk-shaped nucleolus. $\times 5040$.—Fig. 35. Discrete chromatic bodies in matrix below ring-shaped nucleolus. $\times 5040$.—Fig. 36. Section of sporangium with nuclei in metaphase. $\times 1680$.—Fig. 37-39. Metaphase nuclei, showing persistent nucleolus near the equatorial band of chromosomes. $\times 5040$.

Fig. 40-48.—Fig. 40. Sporangium with two exit papillae. $\times 1260$.—Fig. 41. Sporangium with three exit papillae from fixed and triple stained material. $\times 1260$.—Fig. 42. Invaginated exit papilla of fixed and stained sporangium. $\times 3360$.—Fig. 43. Swelling of an exit papilla, with hyaline material beneath it expanding. $\times 3360$.—Fig. 44, 45. Discharging sporangia fixed in osmic acid and stained with a lacto phenol preparation of acid fuchsin-cotton blue. $\times 1260$.—Fig. 46. Elongated spore mass surrounded apically by films of the sporangial hull (fixed and stained as above). $\times 1260$.—Fig. 47.—Separation of layers around sporangial base prior to spore discharge. $\times 840$.—Fig. 48. Empty sporangium with basally separated layers and rhizoidal axis drawn part way up into the sporangium. $\times 3360$.

Fig. 49-61.—Fig. 49-52. Development of resting spore. $\times 1680$.—Fig. 53. Stained resting spore. $\times 1680$.—Fig. 54. Swelling resting spore hull with radiations perpendicular to the concentric zones. $\times 1680$.—Fig. 55-57. Germination of resting spore with hull deliquescing. $\times 1680$.—Fig. 58. Germinating resting spore stained with ruthenium red. $\times 1680$.—Fig. 59. Resting spore shown in figure 58 prior to staining. $\times 1680$.—Fig. 60. Resting spore with one zone of hull differentiated into blunt spines. $\times 1680$.—Fig. 61. Resting spore with vestigial exit papillae. $\times 1680$.

gelatinous hull expands, the sporangial contents undergo the characteristic changes observable in most chytrids. Refractive globules appear in the cytoplasm and coalesce to form larger ones, and at the same time additional small globules continue to form (fig. 23). When the process of accumulation of refractive material is completed, the sporangial contents appear dingy grey or greyish-brown (fig. 24). Following this stage, the refractive material gradually becomes dispersed again so that the protoplasm appears more evenly greyish-granular (fig. 25). The tiny granules then aggregate in groups (fig. 26), and the sporangial contents suggest the "alveolar stage" described by Berdan (1941) for *Catenochytridium carolinianum*. As the sporangial contents continue to mature, the tiny granules coalesce to form the definitive globules of the zoospores, and as this takes place, the sporangial protoplasm gradually becomes less granular and more optically homogeneous. Subsequently, cleavage furrows become visible and delimit the polyhedral zoospore initials (fig. 27).

Fixed and stained preparations show that the young incipient sporangium is uninucleate (fig. 28, 29), and as a rule it remains in this state until the sporangium has attained its maximum size. As the young sporangium grows, its nucleus increases tremendously in size. To determine the magnitude of nuclear enlargement, measurements were taken of many nuclei and their respective sporangia. In young sporangia 8.3 to 11.2 μ in diameter, the primary nucleus was found to be 1.5 to 2.2 μ in diameter, but in sporangia 25.4 to 33.6 μ in diameter the primary nucleus was 6.0 to 9.7 μ in diameter. This shows an approximate increase in nuclear size of 4.5 to 8.2 μ . This increase may be gauged visually by comparing figures 29, 30, and 31, which are of the same magnification. The sporangia, from which the primary nuclei shown in figures 30 and 31 were drawn, could obviously not be shown at the same magnification, since just one of them would practically cover the entire plate.

Densely staining granules usually are present in the cytoplasm around the primary nuclei (fig. 28-31), but similarly staining chromatic granules also occur in other regions of the cytoplasm (fig. 28). The perinuclear granules may represent nuclear extrusions. However, the presence of similar granules in other regions of the cytoplasm suggests that not all of these bodies have originated from the nucleus.

In well fixed and stained preparations the prophase stages of division are easily recognizable. They resemble the prophasic configurations found by Karling (1937) and Hillegas (1940) in *Cladochytrium replicatum* and *Endochytrium operculatum*, respectively. During the early prophases the chromatic reticulum is oriented on the nucleole (fig. 32). Figures 33 to 35 are interpreted as later prophase stages. They indicate that the nuclear reticulum (fig. 32) differentiates into elongate nodular chromosomes (fig. 33, 34). In figure 33 the nodular

strands are not associated with the nucleole, but in figure 34 similar strands are oriented on the nucleole. The number of chromosomes has not been ascertained with certainty, but six to eight discrete bodies may be seen in the late prophase stage, as is shown in figure 35.

The nucleole of prophase nuclei is variable in appearance. As shown in figures 32 and 33, it may be a biconcave disk, a monoconcave heterogeneously staining disk as in figure 34, or a ring-shaped structure (fig. 35). These variations lead the writer to believe that the vacuolate nucleole shown in figure 31 represents an early prophase, the vacuolation of the nucleole possibly representing the initial stage of nucleolar differentiation ultimately resulting in a ring-shaped structure (fig. 35). The biconcave nucleoles of figures 32 and 33 in this event are interpretable as stages intermediate between figures 31 and 35.

Nuclear division is mitotic and simultaneous (fig. 36). As in other chytrids so far studied, the division spindle is intranuclear (fig. 36-39). In the equatorial plate stage, it extends across the nucleus as a relatively narrow spindle-shaped structure, its poles terminating in minute dense bodies from which faint cytoplasmic strands may radiate (fig. 37, 38). However, these rays are not as prominent as those found by Hillegas in *E. operculatum*. At this stage the chromosomes are closely crowded, forming a nodular band across the equator and are not readily distinguishable as separate bodies. The nucleole, as in *C. replicatum* and *E. operculatum*, is persistent during division. In *R. coronum* it is even more prominent at the metaphase than in the two previous genera. It lies very close to the equatorial band of chromosomes, and under low magnifications appears to be a part of it (fig. 36). However, at higher magnification it appears distinct from the latter, and in Flemming's triple dye it stains brilliantly red in contrast to the reddish-violet color of the chromosomes (fig. 37-39).

For the discharge of zoospores one to five exit papillae develop. In living material the papillae appear as fairly low, broad projections under which occur slightly triangular hyaline areas (fig. 40). In fixed and stained preparations, on the other hand, the papillae appear concave, as shown in figures 41 and 42. This reversal in shape may perhaps result from dehydration and plasmolysis of the spore plasm whereby the papillae are invaginated. With further development, the wall of the papilla gradually softens and swells (fig. 43), and at the same time the hyaline material beneath it begins to expand and push upward. As the papillar wall deliquesces, the hyaline mass is pushed out by the emerging spores, distends, and surrounds the emerging spores as a thin transparent layer (fig. 43, 44). As more and more spores emerge, it is distended further, and becomes progressively thinner. Very shortly thereafter the spore mass begins to elongate, becoming tear-shaped and pushes through the gelatinous hull around the sporangium. By this time

the hull itself has undergone partial deliquescence, and as the elongate spore mass reaches the surface, the major portion of it breaks off near the sporangium (fig. 46) and floats away.

It was earlier stated by the writer (1944) that the hull usually deliquesces before spore discharge begins, but subsequent studies in conjunction with staining and fixation at the time of discharge have shown that deliquescence at the time of discharge, as described above, is far more prevalent.

If there is more than one exit papilla in *R. coronum*, all function and discharge zoospores simultaneously (fig. 44, 45). Most of the spores float off as elongate masses, as described above, but some remain in the sporangia. The latter swarm within the sporangia but eventually emerge one by one and swim away. The spores that float off in elongate masses remain quiescent for from one to three minutes, and then begin to oscillate. The flagellum is at first tightly wound around the spore body, but as the spore begins to move, the flagellum gradually unwinds. In from four to sixteen minutes the matrix around the spores disappears. The spores, however, do not swim away directly but jerk about and appear to have great difficulty in freeing their flagellae as if the latter were entangled with each other.

In conjunction with spore discharge, a peculiar differentiation takes place at the base of the sporangium. Two lamellae in this region separate, due possibly to a swelling of a matrix between them (fig. 47). The intervening substance has the same staining properties previously described for the gelatinous hull surrounding the sporangium. The basal separation of layers may (fig. 47) or may not (fig. 40, 43) be evident prior to the emission of spores, but it usually becomes prominent as discharge begins (fig. 44-46). The basal mechanism appears to force the spore mass up against the sporangial apex and simultaneously pulls the rhizoidal axis up through the outer layer (fig. 44-47). Whether or not this basal differentiation is essential to spore discharge is not definitely known, but the structure persists in empty sporangia (fig. 48). In old cultures the basal mechanism may be compound, as shown in figure 15, previously referred to in relation to coronal modifications.

Formation and germination of resting spores.

As cultures age, asexual resting spores are formed. In the early stages they are similar in appearance to incipient zoosporangia (fig. 49). However, as development progresses, large amounts of refractive material in the form of globules accumulate in them (fig. 50, 51). The latter coalesce so that eventually one or several large refractive globules are present (fig. 52). While this is going on, the spore wall thickens and becomes golden in color. Simultaneously the gelatinous hull differentiates into two (fig. 50-52) or three (fig. 53) zones, the outermost of which is almost as dense as the sporangial wall (fig. 50-52). Figure 53 drawn from fixed and stained material shows the solitary nucleus of the resting spore.

When germination begins, the zones of the hull swell and become less dense, and sometimes radiations from the sporangial wall perpendicular to the zones and passing through them may be evident (fig. 54). While the hull is gelatinizing and swelling, the sporangial protoplasm undergoes characteristic changes similar to those described for many chytrids. When these changes have been terminated, a small zoosporangium develops apically or somewhat subapically, as shown in figure 55. The hull of the resting spore may during this time deliquesce (fig. 55-57), but the developing zoosporangium is enveloped by a delicate corona of its own (fig. 58). The latter, however, is so delicate that it cannot be seen without the aid of staining with ruthenium red, janus green, or neutral red. Accordingly, it is not shown in figures 55 to 57 drawn from unstained material, but in figure 58, drawn from material immersed in a dilute aqueous solution of ruthenium red, it is clearly visible. Figure 59 represents figure 58 previous to staining. In this instance (fig. 58) the gelatinous hull was found to be intact, unlike those represented in figures 55 to 57.

In addition to the typical resting spores above described, some are occasionally found in which one zone of the hull has become folded around the spore wall (fig. 60). Resting spores showing vestiges of exit papillae in their walls (fig. 61) are also found. The latter indicate that resting spores are encysted sporangia.

Relationships.—This chytrid is unusual in having a gelatinous hull or corona enveloping the zoosporangium and resting spore, but in other characters it is essentially like other species of the genus *Rhizophyidium* in which it has been placed.

R. coronum is the first chytrid with a gelatinous hull found in the New World, but a fungus with a somewhat similar hull was partially diagnosed by Lind in Sweden (1905). Because of Lind's meager illustrations and incomplete diagnosis, Sparrow (1943) included it as a doubtful member of the genus *Rhizophyidium*, expressing doubt that the organism was fungoid in nature. The discovery of *R. coronum*, however, indicates that *R. gelatinosum* (Lind) is most probably a similar fungus, though a marine counterpart, being epiphytic on a marine alga. Despite Lind's incomplete diagnosis, comparison between *R. gelatinosum* and *R. coronum* is partially possible and seems warranted.

The sporangia of *R. gelatinosum* are described as being spherical, 20-30 μ in diameter. If they were all of this shape and size, then our American fungus exhibits greater variability in both size and shape, for in the latter they may be spherical, (11-49 μ in diameter), or ovoid (10-48 \times 14-54 μ in diameter). In both species the sporangial wall is smooth and colorless. The width of the gelatinous hull of *R. gelatinosum* is given as consistently 3 μ , but in *R. coronum* no definite measurement can be given, since it is not a static structure, but expands during growth of the zoosporangium. The number of exit pores given for *R. gelatinosum* is eight, the

greatest number recorded for species of *Rhizophydium*. In *R. coronum* no more than five have been observed, and in this respect it is more like *R. sphaerotheca* (Zopf, 1887) for which two to five exit papillae were recorded. Zoospores, resting spores, and rhizoids of *R. gelatinosum* were not described by Lind. Accordingly, comparison with *R. coronum* regarding these structures is impossible.

SUMMARY

Rhizophydium coronum is saprophytic on decay-

ing vegetable debris in soil. In structure and development it is essentially like other species of *Rhizophydium*, but differs from all known members by developing a gelatinous hull or corona around its zoosporangia and resting spores. Resting spores develop asexually and in germination function as prosperangia.

DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY,
NEW YORK 27, NEW YORK

LITERATURE CITED

- BERDAN, H. B. 1941. A developmental study of three saprophytic chytrids. II. *Catenochytridium carolinianum*. Amer. Jour. Bot. 28: 901-911.
- HANSON, A. M. 1944. Three new saprophytic chytrids. Torrey 44: 30-33.
- HILLEGAS, A. B. 1940. The cytology of *Endochytrium operculatum* (de Wildeman) Karling in relation to its development and organization. Bull. Torrey Bot. Club 67: 1-32.
- KARLING, J. S. 1937. The cytology of the Chytridiales with special reference to *Cladochytrium replicatum*. Mem. Torrey Bot. Club 19: 3-92.
- LIND, J. 1905. Ueber einige neue und bekannte Pilze. Ann. Mycol. 3: 427-432.
- SPARROW, F. K., JR. 1943. Aquatic Phycomycetes. The University of Michigan Press. Ann Arbor.
- ZOPF, W. 1887. Ueber einige niedere Algenpilze (Phycomyceten) und eine neue Methode ihre Keime aus dem Wasser zu isolieren. Abhandl. Naturforsch. Gesell. Halle 17: 77-107.

PLANT NUTRITION IN RELATION TO DISEASE DEVELOPMENT.

II. CABBAGE CLUBROOT¹

J. C. Walker and W. J. Hooker

IN A previous paper (Walker and Hooker, 1945) the writers reported a study of the relation of plant nutrition, temperature, and inherent host resistance to the rate of development of cabbage yellows (*Fusarium oxysporum* f. *conglutinans* (Wr.) S. & H.). In a susceptible variety of the host the disease developed more slowly at 19°C. than at 25°, and at the lower temperature it was suppressed still more with increase in salt concentration of a balanced nutrient solution. At 25° the effect of nutrient was in the same direction but less marked. In a variety with a moderate degree of inherent resistance the disease developed very slowly at 19° and no measurable effect of nutrient was observed. At 25° the disease developed at about the same rate and with the same interacting effect of nutrient as in the susceptible variety at 19°. Omission of potassium in the nutrient tended to increase the disease rate while omission of either nitrogen or phosphorus tended to reduce it.

Cabbage yellows is a typical vascular *Fusarium* disease in which toxic materials produced in the invaded xylem vessels are transported throughout the plant and a systemic toxemia brings on stunting, chlorosis, leaf drop, vascular necrosis, and

death. It may be classed as a hypoplastic disease. The clubroot disease (*Plasmodiophora brassicae* Wor.) of the same plant causes quite distinct host reactions. Invasion by the parasite results in hyperplasia and hypertrophy of the parenchymatous root tissue and in suppression of cell differentiation. This disease may be classed as hyperplastic.

In this laboratory, Pryor (1940) studied the relation of nutrients to the development of clubroot. He used a one-tenth dilution of Hoagland's solution identical in formula and concentration with that designated as 0.1H in the study of cabbage yellows (Walker and Hooker, 1945) and increased or omitted the K and NO₃ ions. He found that the percentage of infected cabbage plants was in general increased slightly over that of the balanced solution with an increase in nitrogen and in potassium, and with the omission of nitrogen, but the percentage of infected plants was markedly decreased with the omission of potassium. Inasmuch as these results indicated a response to nutrition at variance with that of yellows, the present investigation was undertaken to study further the effect of ion balance and also that of salt concentration upon disease development.

METHODS.—The method of plant culture and the nutrient solutions used were similar to those used for yellows (Walker and Hooker, 1945). Clubs were collected from naturally infected cabbage plants in the field, washed, frozen, and stored at -10°C. Inoculum was prepared by macerating clubs with water in a Waring blender. The coarse debris was

¹ Received for publication June 25, 1945.

Investigation supported jointly by the Department of Plant Pathology, University of Wisconsin, with the aid of a grant from the Wisconsin Alumni Research Foundation, and by the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

removed by centrifuging the suspension in a Sharples centrifuge. The spore mass was suspended in water and centrifuged three successive times. The final suspension was adjusted to contain about 65,000 spores per mm³. To each pot of quartz sand in which plants had been transplanted, 100 cc. of spore suspension was added. Two to four replicates of each treatment were made and the order of pots was randomized.

Seed was sown in quartz sand watered with the basal solution (1H) and plants were transplanted to the pots two to three weeks later. As soon as the plants had recovered from transplanting, the test solutions were started and continued throughout the duration of the experiment. The reaction of the solutions as they drained from the pots was tested from time to time and the pH found to be within the range favorable for clubroot infection. Inoculations were made about two weeks after test solutions were started. Plants were grown for 30 to 68 days after inoculation. They were then removed and placed in one of four classes designated according to the degree of disease development as (1) free from clubs, (2) slightly diseased, (3) moderately diseased and (4) severely diseased. A disease index was calculated for each pot as follows: the number of plants in class 1 was multiplied by 0, that in class 2 by 1, in class 3 by 2, in class 4 by 3. The sum of the products multiplied by 100 was divided by 3 × total plants to secure the index. Thus the indices secured might range from 0, indicating all free from clubs, to 100, indicating all severely clubbed.

RESULTS.—In the first three experiments, concentrations of 1/10 basal (0.1H), basal (1H), two times basal (2H), and three times basal (3H) were used. At each concentration a +K solution was used in which the concentration of the potassium ion was increased by 28 per cent, and a —K solution in which the potassium ion was omitted after the plants had been inoculated. The results are given in table 1.

The first experiment was run from December 13 to February 19, a period of 68 days, during which light intensity was low and days relatively short. It is to be seen that the index was significantly lower at each concentration in the —K solution. There was no appreciable difference between the indices in the balanced and in the +K solutions. The indices rose with increase in concentration in both the balanced and the +K solutions. The only marked increase in the —K solution was at the 3H level. However, increases due to increase in concentration were usually not significant.

The second experiment was run from March 17 to April 16, a period of 30 days. With increasing light intensity and day length, growth was more rapid than in the first experiment. The results showed again markedly less disease development in the —K solution. With the exception of the index at 1H in the +K solution, there was a tendency in balanced, +K, and —K solutions for the disease

TABLE 1. *Relation of salt concentration and presence or absence of potassium to the clubroot index.*

Expt. number and date	Nutrient conc.	Disease index in		
		Balanced sol.	+K sol.	—K sol.
1 ^a December 13 to February 19	0.1H	62	58	21
	1H	70	79	20
	2H	80	84	22
	3H	82	90	34
	0.1H	37	39	2
2 ^b May 17 to April 16	1H	52	27	24
	2H	67	70	49
	3H	62	75	41
	0.1H	37	41	39
3 ^c April 22 to June 1	1H	73	73	47
	2H	69	74	39
	3H	83	94	54

^a Least significant difference between treatments (19:1): 24.3.

^b Due to variability between replicates treatment differences could not be detected by statistical analyses.

^c Least significant difference between treatments (19:1): 32.8.

index to increase from 0.1H up to 2H, but little difference was evident between 2H and 3H. The variability between replicates in this experiment prevented demonstration of treatment differences by statistical analysis.

The third experiment ran from April 22 to June 1, a period of 40 days, during a time when light conditions were even more favorable for growth. There was no appreciable difference in disease between balanced, +K, and —K solutions at 0.1H, where the total amount of growth of the host was least. At 2H and 3H the indices in the —K solution were in each case significantly lower than they were in the +K solutions. In the balanced and +K solutions the indices were significantly lower in the 0.1H solution than in either of the three highest concentrations, but differences between the last three mentioned were not great.

The results of the three experiments indicated that in young plants clubroot developed best in a balanced solution or in one in which potassium was increased, while omission of potassium reduced disease development. The differences between the —K solution and the other two solutions tended to be less in the second and third experiments when light conditions favored most rapid growth of the host plants.

The above experiments indicate a suppression of disease in the absence of potassium and a tendency for the growing conditions to influence the results. During the following season, two experiments were conducted at the 2H concentration. Experiment 4 was run from November 23 to January 5, a period of 43 days, when light intensity was low and days short. Experiment 5 was run from March 10 to April 20, a period of 41 days, when light intensity and day length were more favorable for growth.

TABLE 2. *Relation of salt concentration and balance to the clubroot index. Experiments 4 and 5.*

Nutrient balance (2H conc.)	Experiment 4			Experiment 5	
	Ave. weight of uninoc. plants grams	Yellows index	Clubroot index ^a	Ave. weight of uninoc. plants grams	Clubroot index ^b
Balanced	6.84	53	50	20.47	78
+N	8.34	46	67	20.68	93
—N	1.97	42	80	1.25	91
+P	7.56	48	59	14.35	80
—P	7.82	41	23	15.37	60
+K	8.53	58	64	24.82	94
—K	6.86	83	42	4.84	88

^a Least significant difference between treatments (19:1):22.2.^b Least significant difference between treatments (19:1):18.1.

The balanced solution was compared with solutions in which potassium, nitrogen, and phosphorus, respectively, were omitted, and with solutions in which nitrogen, phosphorus, and potassium were increased above the balanced solution at the ratios of 1.15 to 1, 2.0 to 1, and 1.28 to 1, respectively. In experiment 4 a parallel series with yellows was added. Parallel uninoculated pots were included and at the end of each experiment the fresh weight of the tops of healthy plants was determined. The results of the two experiments are given in table 2.

In the balanced solution the weight of tops in experiment 5 was approximately three times that in experiment 4, reflecting the much better growing conditions in the former. In experiment 4, statistical evaluation indicated no significant difference in amount of growth in the various solutions except in the —N solution. In experiment 5, growth in both the —N and —K solutions was distinctly less than in the remaining solutions.

Insofar as yellows was concerned, the greatest effect was that of significant increase in the disease index in the —K solution. Decreases in —N and —P solutions occurred but they were less pronounced. In the same series clubroot was enhanced slightly in +K, more so in +N, but most in —N solution where the index was significantly higher than in the balanced solution. It was suppressed in —K and significantly so in —P. With respect to N and K, this was in accord with the results of Pryor (1940). In experiment 5, the disease advanced generally much farther in a 41-day period than in the 43-day period of experiment 4. Excess nitrogen and absence of nitrogen caused higher indices. The difference between +K and —K was reduced to a negligible amount. The —P solution again suppressed disease development appreciably. As indicated previously in experiment 3, the better conditions of growth tended to reduce the differential effect of various solutions.

Discussion.—In this investigation the balance of nitrogen and potassium was varied in the same manner as in the study reported by Pryor (1940). However, the concentration of salts used was 20 times higher than in the earlier work, and a disease

index instead of percentage of infected plants was employed as a measure of disease development. The results of the present investigation confirm those of Pryor insofar as effect of nitrogen and potassium on clubroot development is concerned.

In view of the fact that yellows and clubroot involve quite distinct types of relation between the same host and two quite different parasites, it is of interest to compare the results presented in this paper with those presented earlier in a similar investigation with yellows (Walker and Hooker, 1945). Increase in salt concentration retarded yellows and enhanced clubroot. Excess nitrogen increased both diseases; lack of nitrogen increased clubroot but decreased yellows. Excess of potassium enhanced clubroot development but had no appreciable effect upon yellows. Lack of potassium had a profound influence upon both diseases but affected each differently; yellows was decidedly increased, while clubroot was markedly retarded. Excess phosphorus had little effect on either disease; lack of phosphorus retarded both yellows and clubroot.

It would appear that the absence of potassium or excess of nitrogen in the nutrient solution either enhanced the pathogenicity of the yellows organism in the xylem vessels or increased the susceptibility of the host to the toxic materials produced in the xylem; increase of the concentration of the balanced solution in general had an opposite effect.

In the case of clubroot, increase in disease severity is dependent upon increased growth activity in infected meristematic tissues. It might be expected, therefore, that nutritional effects which increased growth would increase the disease index. There was such a correlation when growth increased as a result of greater salt concentration. However, it is clear that the influence of nutrition on clubroot is not merely an influence upon growth rate of the host. Omission of nitrogen suppressed growth of the plant most promptly and extensively of all nutrient variations used, but it also resulted in clubroot enhancement. On the other hand, omission of potassium, although it reduced growth, usually resulted in clubroot suppression. It was also evident

that when length of day and light intensity were most favorable for growth of cabbage seedlings the differential effect of salt balance was less pronounced. The effects of nutrient upon disease expression in plants are varied and so far not readily explained. They are probably exerted upon the host and pathogen separately as well as upon the interaction of the two organisms.

SUMMARY

The development of clubroot in young cabbage plants was studied in relation to salt concentration and balance in the nutrient solution.

Increase in salt concentration in a balanced solution tended to increase the disease index.

Excess of potassium and excess of nitrogen increased the disease index; increase of phosphorus had little effect.

Omission of potassium or phosphorus usually decreased, while omission of nitrogen increased the disease index.

When light conditions and salt concentration were favorable for good growth of the host, the effect of salt balance was less evident than under conditions in which the plants grew more slowly.

DEPARTMENT OF PLANT PATHOLOGY,
UNIVERSITY OF WISCONSIN, AND
DIVISION OF FRUIT AND VEGETABLE CROPS AND
DISEASES,
BUREAU OF PLANT INDUSTRY, SOILS, AND
AGRICULTURAL ENGINEERING,
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
MADISON, WISCONSIN

LITERATURE CITED

- PRYOR, DEAN E. 1940. The effect of some mineral nutrients on the development of clubroot of crucifers. *Jour. Agric. Research* 61: 149-160.
WALKER, J. C., AND W. J. HOOKER. 1945. Plant nutrition in relation to disease development. I. Cabbage yellows. *Amer. Jour. Bot.* 32: 314-320.

CELL ELONGATION AND THE DEVELOPMENT OF ROOT HAIRS IN TOMATO ROOTS ¹

R. G. H. Cormack

IN PREVIOUS papers, the present writer (Cormack, 1935, 1937, 1944) studied the effect of environmental factors on root-hair development and the physical and chemical changes produced in the walls of the elongating epidermal cells. The last paper (1944), considered also the emergence of a hair and its final position in the mature cell. With regard to this particular aspect of the problem several observations and opinions have been reported. Snow (1905) has found that in corn the hair originates near the tip of the root where the cells are isodiametric. Nearly the whole wall curves at first, but with the continued stretching of the cell this primary bulge becomes a papilla. Roberts (1916) has shown that in endive and lettuce roots grown in moist air the initial formation of the root hair is indicated by a general swelling of the outer wall of the epidermal cell and that further swelling, followed by growth, takes place at the less resistant portion of the wall. Cormack (1935) has concluded that in the formation of a hair in cabbage and white mustard roots, the wall is pushed out at its softest point invariably at or near the apical end of the cell. The extreme shortness of these cells indicates that longitudinal growth has ceased by the time the hairs start to grow.

In their study of cell polarity and the differentiation of root hairs in related genera of the grass family, Sinnott and Bloch (1939) have assumed that there is a strong apical tendency in the formation of root hairs in the epidermal cells of *Phleum* and a very much weaker tendency in *Sporobolus*. Brum-

field (1942) has confirmed this constant apical position of the hairs in *Phleum* roots. The present writer (1944) investigated again the development of hairs in *Phleum* and *Sporobolus*. In *Phleum* the hair originates as a narrow bulge at the apical end of the cell and remains there. On the other hand, in *Sporobolus* the hair originates as a wide bulge directly at or very near the apical end of a young cell, while in a mature cell its position is more central. All evidence indicates that the cells are still growing in length in the hair-producing region of *Sporobolus* roots.

The present paper reports the results of a study planned to throw more light on how this change in position of the papilla takes place and the ability of a papilla to grow into a hair. It also includes a further study of the effects of environmental factors on elongating cells.

For the present study roots of tomato seedlings were chosen as the experimental material, for these roots are similar to *Sporobolus* in their root-hair development (Cormack, 1935) and, being larger, are much easier to study.

EXPERIMENTS AND RESULTS.—*The origin and development of root hairs in moist air.*—Seeds of tomato (*Lycopersicum esculentum* Mill.) germinate readily between moist blotters at room temperature (22°C.). The roots grow rapidly and when they have reached a length of 1.5-2 cm., are stained in methylene blue for a few minutes and then examined.

A preliminary examination has confirmed the observations of the earlier study, that the epidermal cells are all of one kind, that any cell may form a hair and that there is a mingling of papillae and

¹ Received for publication February 27, 1945.

hairs of varying length in the mature region. However, the epidermal cells are long and since many are hairless, the hairs appear to be sparsely distributed over the whole surface. Roberts (1916) has reported the mingling of papillae and hairs when roots of alfalfa, cabbage and *Verbascum* are grown in moist air, but no definite conclusions have been reached as to its cause.

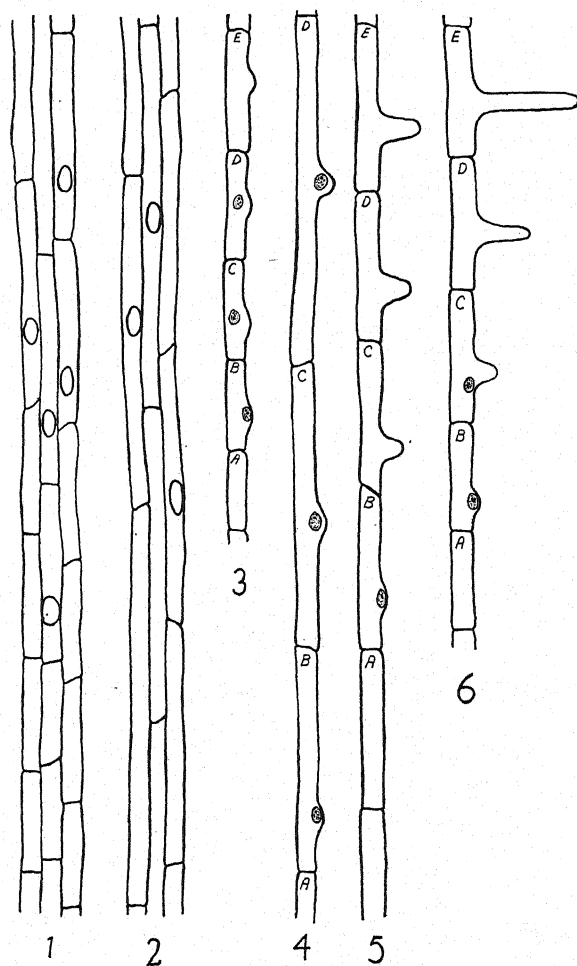


Fig. 1-6. Strips of epidermis from roots grown in moist air. The root apex is toward the bottom of the page in all figures. Circles represent bases of papillae; all figures $\times 150$.—Fig. 1. Immature region of the root, the papillae emerging near the apical ends of the cells.—Fig. 2. Mature region of the same root shown in figure 1, with the papillae in the middle of the cells.—Fig. 3-6. Individual rows of cells from different roots, viewed from the side to show a series of successively older cells A-E, with B the first papilla-producing cell in each row. The position of the nucleus is indicated.—Fig. 3, 4. Successively older cells C, D and E show a marked increase in total cell length, with a corresponding increase in the apical portion of the cell wall over that of cell B, with little change in the basal portion. The bulge remains a papilla.—Fig. 5, 6. Successively older cells C, D and E show little increase in total cell length and length of the apical portion, and the papilla grows into a hair.

A more critical examination has yielded results which are best presented by a series of drawings. Figure 1 represents the immature region of the root where the papillae first make their appearance. A papilla emerges as a wide bulge measuring $18-32 \mu$ in diameter near the apical end of a cell. As in *Sporobolus*, however, due to the wide nature of the bulge, care must be taken not to confuse papillae that are just emerging with those that have already emerged but have stopped growing. This is particularly difficult to determine in roots where the smooth zone preceding the region of first-formed papillae is greatly extended and where very few papillae develop. In roots of this kind all the epidermal cells are extremely long and the first sign of a papilla occurs much nearer the middle of the cell. Figure 2 represents the mature condition of the same root illustrated in figure 1. Here all the cells are very much longer and the papilla occurs near the middle of a cell. That the papilla-bearing cells continue to grow in length for some time, can be determined by measuring various parts of the outside walls of both fully-grown cells and cells that are just beginning to form papillae. The usual procedure is to measure one hundred immature and one hundred fully-grown cells from ten roots and thus to determine the average increase in length. In one such instance the fully-grown cells show an average increase in total cell length of 60μ , of this the apical end (below the papilla) increases 51μ and the basal end (above the papilla) 9μ . From these results it is clear that a cell continues to grow in length after a papilla begins to push out and that growth occurs mainly at the apical end. In some roots where longer hairs develop, the condition of the mature region is much the same. In other roots, hairs are more densely developed for a short distance at the very top. In this region the cells are much shorter than those developed later and almost every cell forms a long hair.

To study the growing papilla-forming cells more closely a series of four or five successive cells in individual rows are measured. For this study only rows are selected where each successive cell of the series has formed a papilla and where it is apparent that all the cells under observation are growing at an approximately uniform rate. Figures 3-6 illustrate typical rows of this kind. The youngest cell is marked A, the first cell to produce a papilla is marked B, and successively older cells C, D, and E. In the four typical examples illustrated the papilla is seen to emerge as a wide bulge near the apical end of cell B. Successively older cells C, D and E in figure 3 and C and D in figure 4 show a marked increase in total cell length and a corresponding increase in length of the apical portion of the cell wall over that of cell B, with little change in length of the basal portion. In each of these older cells the papilla fails to grow into a hair and assumes a more central position. The reverse is found in figures 4-5. Here successively older cells C, D and E show little or no increase in total cell length over that of cells

A and B. In each of these older cells the papilla grows into a long straight hair in almost its original position. This is most noticeable in cell E (fig. 6) where growth of the hair is very rapid. The base of the hair is wide and sloping and the hair itself grows straight out at right angles to the axis of the root. Hairs of this kind have been described in *Sporobolus* roots (Sinnott and Bloch, 1939; Cormack, 1944).

The weight of evidence is for continuation of elongation of a papilla-producing cell and for more rapid growth in the apical end of the cell than in the basal end. Accordingly the final position of the mature papilla or hair will depend on the length of time the apical end continues to grow. If this period is short the original apical position will be more or less maintained, if greatly protracted, the papilla will be found in the middle and occasionally in the basal portion of the full-grown cell. The length of time elongation continues also determines whether the papilla remains or whether it grows into a hair. Such may be the explanation of the mingling of papillae and hairs of varying length in the mature region of tomato roots.

Presumably the length of time during which individual cells continue to elongate will depend on differences in metabolic activity, differences in response to environmental conditions and differences in intercellular relationships. Concerning the influence of the growth of one cell on another, Snow (1905) has concluded that the lagging behind of the inner cells of the cortex in corn roots during the elongation period allows the papilla to become a hair. This theory has been strongly supported by Jeffs (1925). More recently Sinnott and Bloch (1939a) in their study of differential wall growth in *Phleum* roots have been able to show that the cells do not slide along one another, but are held so firmly together that points in adjacent cell walls which are originally opposite each other are still opposite after these cells have greatly elongated. Moreover they have shown that those portions of adjacent cells opposite the slowly growing trichoblasts do not grow as fast as the parts opposite their faster growing sister cells.

From the results of the present study, the writer is of the opinion that as individual cells grow differentially they exert an influence upon the growth of neighboring cells. Some evidence of this is obtained by examining the arrangement of fully-grown cells in the mature region of the root. In general, the apical end of a mature papilla-bearing cell is longer when adjacent to hairless cells than when adjacent to other papilla or hair-bearing cells. Similarly the apical end is longer when opposite the apical end of an adjacent papilla-bearing cell than when opposite the basal end of the same kind of cell. He also thinks, that though individual cells grow differentially, all the surface cells in the same region of the root elongate at a uniform rate. Some evidence in support of this viewpoint is obtained by examining roots with alternating zones of longer and shorter cells. In each zone, though the cells vary in length all the cells are noticeably longer or shorter

than those in the zone either directly above or below. It has also been shown (Cormack, 1935, 1944) in roots where zones of longer and shorter cells are produced by suddenly changing the environmental conditions that all the cells at the same level react at the same time to the new conditions.

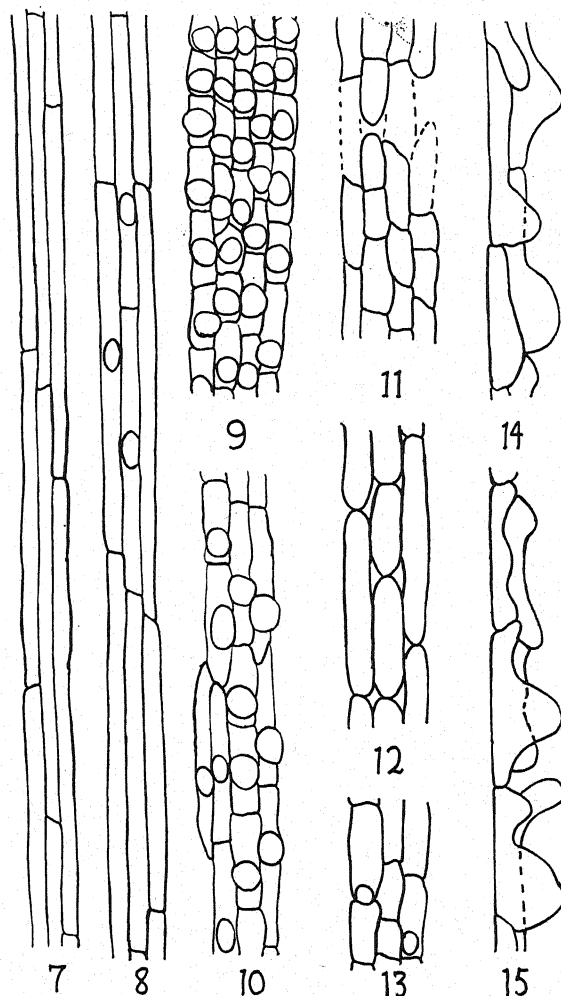


Fig. 7-15. Strips of epidermis from the mature region of roots developed in various experimental solutions. The root apex is toward the bottom of the page in all figures; all figures $\times 150$.—Fig. 7, 8. Roots developed in tap water; all cells extremely long.—Fig. 7. Hairless type of root.—Fig. 8. Root with scattered papillae, which occur in the middle of the cells.—Fig. 9, 10. Roots developed in a neutral, saturated CaSO_4 solution. Circles represent hair bases. In figure 10, all the cells are longer and produce shorter hairs.—Fig. 11-13. Roots developed in slightly alkaline CaSO_4 solutions, showing mild injury of the epidermis.—Fig. 11. Hairless; the walls of some cells are broken (indicated by dotted lines).—Fig. 12. Hairless; cells pulled apart at the corners to form diamond-shaped and triangular intercellular spaces.—Fig. 13. Cell walls intact; a few stunted hairs, found at the apical ends.—Fig. 14, 15. Various forms of abnormal cells produced in slightly acid distilled water. Swelling and separation of the cells indicating lack of stability in the primary wall.

TABLE 1. *Measurements of the length of different parts of epidermal cell walls and of hairs grown in various experimental solutions. All the measurements are from the mature region of each root and represent the average of the cells illustrated in the figures referred to.*

Experimental solution	Length of apical end in microns	Diameter of papilla or hair base in microns	Length of basal end in microns	Total length of cell in microns	Length of hairs in microns
Tap water, hairless root (fig. 7).....	298
Tap water, hairless cells (fig. 8).....	319
Tap water, papilla-producing cells (fig. 8)...	101	24	123	248	papillae
Saturated CaSO ₄ (fig. 9).....	11	21	14	46	1300-1800
Saturated CaSO ₄ (fig. 10).....	26	27	48	101	300-450

Another point of interest is the position of the nucleus in the papilla-producing cell. Haberlandt, according to Farr (1927), has concluded that the nucleus lies along the exterior wall in the vicinity of the incipient protruberance. Windel, according to the same authority, and Farr himself (1925), have corroborated Haberlandt's findings, namely, that the nucleus in many species follows along at a rather uniform distance behind the growing hair tip. Later, Farr (1928a) was of the opinion that the nucleus interferes with the streaming of the protoplasm in duplex hairs and thus retards growth of the hair. Previous to this, Roberts (1916) had reported no relation between the position of the nucleus and the formation of a hair. In the present study the nucleus is found in the vicinity, and is frequently closely appressed to the exterior wall of the developing papilla (fig. 3-6), thus corroborating Haberlandt's observations. How it influences the initiation of a papilla is still a matter of conjecture.

Whether root hairs develop in a similar manner in the soil is not known. If they do, it is not likely that serious rupturing of the hairs occurs, for when the surface cells are still elongating the hairs are mainly papillae and when longer hairs develop longitudinal growth has practically ceased.

The origin and growth of root hairs in aqueous solutions.—The next series of experiments are designed to produce changes in root-hair development by manipulation of the amount of calcium and of the hydrogen-ion concentration of the aqueous solution. In a long series of papers already listed in the writer's earlier work, Farr (1925-1928) and Mrs. Farr (1927-1929) have shown that calcium and a suitable pH are indispensable for the elongation of root hairs in aqueous solutions. Later it was shown (Cormack, 1935) that calcium fails to unite with pure pectic acid in an acid medium and that in neutralizing the pectic acid there may be weak or strongly calcified pectates. Subsequent experimentation with living roots at that time and later (Cormack, 1944) has shown that the amount of calcium and the pH of the solution modifies the rate at which calcification of the cell walls takes place and thus determines the capacity to produce root hairs, and also determines the shape and length of the hairs themselves.

Earlier tests with tomato roots (Cormack, 1935) has shown that in tap water all the epidermal cells are long and the hairs are sparsely developed. Slightly alkaline calcium salt solutions produce short cells, every one forming a long hair. However, the results already obtained necessitate a more critical study of the reaction of the epidermal cells to various solutions.

Young seedlings with roots about 2 mm. long are mounted in holes in a thin sheet of cork. The cork bearing the seedlings is then floated on the surface of the experimental solution, in a small dish, the roots hanging vertically downwards. Once again the results of the experiments can best be presented by a brief description of the epidermis, with reference to illustrations of rows of cells (fig. 7-15) and measurements which are recorded in table 1.

Hairs are sparsely developed in tap water, thus confirming the observations of the earlier study. Although hairs are formed for a short distance near the top, the roots soon become hairless (fig. 7) or papillae and very short hairs develop (fig. 8). The general effect is to favor elongation of the epidermal cells and to suppress development of papillae and hairs. Where papillae develop (fig. 8), they are found well toward the middle of the mature cells. The extreme length of both hairless and papilla-bearing cells is shown in table 1. In some roots the cells are not as long as those illustrated, but the general condition of the epidermis is the same.

The best development, and the longest hairs are produced in a saturated solution of calcium sulphate in distilled water at about neutrality. The most striking and uniform results are obtained by allowing the roots to grow in tap water for about ten hours before transfer to the calcium solution. The roots are examined after they have reached a length of 8-15 mm. Many roots are densely haired from the top to the tip, while other roots show zones of denser and sparser hairs. The general effect is to stimulate the production of hairs and to increase their length (fig. 9, 10 and table 1). In figure 9 the cells are so short that the hair base occupies the greatest part of the outside wall, usually at the apical end. In figure 10 all the cells are longer and they produce much shorter hairs. That cell division is also stimulated in the meristem is evident by the increase in the number of cell rows.

Tomato roots are found to be exceedingly sensitive to changes in pH. By slightly increasing the alkalinity, root-hair development is noticeably retarded until at a pH of about 8 the roots become hairless, with or without injury to both epidermis and cortex (fig. 11-13). In figure 11, the outer walls of a few cells are broken. In figure 12, the cells are pulled away at the corners to form diamond-shaped and triangular intercellular spaces. This condition of the epidermis is in sharp contrast to the compact arrangement of the epidermal cells under normal conditions. Similarly shaped intercellular spaces have been demonstrated (McPherson, 1939) to arise in the meristem of corn roots through a pulling apart of the cortical cells at the corners. More recently, Hulbary (1944) has described their origin in the stem tips of *Elodea*. In figure 13, no injury occurs and an occasional cell forms a stunted hair at the extreme apical end.

In still more alkaline solutions with a pH of 8 to 10, very severe injury occurs. All the cells are extremely short and both epidermis and cortex are so badly ruptured that it is impossible to make accurate drawings. The differential effect of the pH of the solution on the stele and on the cortex has been pointed out by Farr (1928). In alkaline solutions the bundle continues to elongate, rupturing the cortex, while in very acid solutions the cortex frequently becomes wrinkled and pulls away locally from the bundle. As recorded previously (Cormack, 1935, 1944), the rupturing of the cortex is attributed to the too-rapid change of the pectic acid lamellae of the elongating epidermal and cortical cells into a strongly calcified pectate. The results obtained with tomato roots in alkaline calcium solutions serve to strengthen that viewpoint.

A noteworthy feature of many roots grown in alkaline calcium solutions is the peculiar condition of the root cap. In these roots many root-cap cells are found adhering to the epidermis over wide areas. In other roots the root-cap cells are found hanging together in long sheets curled forward over the apex of the root. These two conditions of the root cap are in sharp contrast to that in tap water, where the root-cap cells slough off gradually behind the root apex. This peculiar condition of the root-cap cells suggests that the pectic acid of their middle lamellae has become strongly calcified.

The next series of experiments are designed to test the effect of calcium deficiency and acidity of the aqueous solution. For this study seedlings are grown in distilled water with a pH of 6.0 to 6.6. The roots grow poorly and the general effect is to suppress or inhibit completely the development of hairs. Some roots produce a scattering of papillae and short hairs but the majority are hairless. In these roots a few large, swollen cells are occasionally observed among the papilla-forming cells. By adding very small amounts of hydrochloric acid to the distilled water, abnormalities of this kind are increased, until at a pH of about 5 the roots fail to grow. The appearance of these roots is not uniform. In some the epi-

dermis is obviously swollen and deformed while in others it is smooth and hairless. The abnormality of the mature tissue is due to the effect of the solution on the elongating cells (fig. 14, 15). In figure 14, almost every cell is swollen, particularly at the apical end, while in figure 15 not only are the cells swollen, but some are separated at their end walls. These abnormalities can be explained on the basis of internal pressure on a wall that has been unequally hardened. In this regard it is interesting to note (fig. 14, 15) that some cells are able to affect hardening of the basal portion of the exterior wall, with only the apical end becoming swollen, while other cells are not able to do so and thus the whole exterior wall is pushed out.

It may be seen from figures 7, 8, 9, 10 and from table 1, that there is a definite relationship between epidermal-cell length and length of the full-grown hairs. The longest cells are hairless, somewhat shorter cells may form papillae, while the shortest cells produce hairs nearly 2 mm. long.

These results would seem to indicate that each epidermal cell has a certain capacity for growth which may be expressed either in a longitudinal or horizontal direction. This difference in cell length is based on the rate at which calcification of the cell walls takes place. It is conceivable that if it takes place too slowly (fig. 7), the soft, plastic nature of the middle lamellae will allow the cells to elongate rapidly and thus no hairs will form. If it takes place much more rapidly (fig. 9, 10), elongation will be arrested while the cells are still short and, due to increasing pressure from within, a papilla will be pushed out and will grow into a long hair. The occurrence of papillae in the mature region (fig. 8) represents an intermediate condition between these two extremes.

The experiments with tomato roots in various solutions show that these roots are extremely sensitive to changes in pH and that, in general, hairs are sparsely developed. In this regard they differ quite markedly from roots of cabbage and *Phleum* (Cormack, 1935, 1944) which produce dense hairs in aqueous solutions and withstand a comparatively wide range in pH. This difference in root-hair production is attributed to differences in the character of the epidermis. For example, in cabbage and *Phleum* roots the epidermal cells are differentiated in the meristem into short and long cells. In the former plant, rows of short cells alternate with rows of long cells, while in the latter, short cells alternate with long cells in the same row. In both cases, the short cells are more densely protoplasmic and under most conditions are able to bring about the gradual change from pectic acid to calcium pectate in their cell walls at an early stage and thus form hairs. The long cells are more acid and their walls are not calcified at this stage, thus they remain hairless. It is only under limited conditions that the long cells, with their acidity neutralized, produce long hairs. On the other hand, the epidermis of tomato roots is not differentiated into long and short cells. All the

cells are of one kind and although they are all long, they vary in length in the same region under the same conditions. The length of the cells and their highly vacuolated condition in the elongating zone suggest that they are all of the "long-cell type." The relatively poor development of hairs in aqueous solutions under most conditions and extreme sensitivity to changes in pH substantiate this viewpoint. The similar condition of the epidermis in *Sporobolus* suggests that the same conclusions are also valid here.

DISCUSSION.—Within recent years the development of special techniques and the use of the horizontal microscope have enabled a number of investigators to view directly the growth of living epidermal cells. In this way, both Jeffs (1925) and Farr (1928b) were able to watch the elongation of living root hairs. Jeffs has observed in roots of corn, radish and mustard growing in moist air that there is an overlapping of the region of root hairs with the region of elongation. As a result the emerging root hairs are carried along laterally for a few hours. The rate of root-hair elongation gradually increases as lateral movement of the hair is retarded. Farr has found that root hairs of Georgia collards in water elongate at a constant rate from the start, thus indicating that the epidermal cells or root have already ceased elongating in the region which is producing root hairs.

By similar methods, Sinnott (1939) and Sinnott and Bloch (1939, 1939a) have watched the division and subsequent growth of epidermal cells in delicate grass roots. It has been shown that in individual epidermal cells of *Phleum* roots the end of the cell toward the base of the root attains its final size sooner than the apical end of the same cell. By improving this technique, Brumfield (1942) has shown also for *Phleum* roots that different parts of the same cell may be growing at quite different rates at the same time. In one case in particular, the basal end of an epidermal cell stops growing first, the middle part next and the apical end last, the cessation of growth passing along the cell from base to apex.

Evidence obtained in a previous study with *Sporobolus* roots (Cormack, 1944) has indicated that an epidermal cell may continue to grow after a papilla begins to push out and that as a result the papilla may occupy a more central position in the full-grown cell. In the present study with tomato roots, measurements which have been made of papilla-bearing cells not only substantiate this viewpoint but for the first time show that the apical end of the cell continues to grow after the basal end has stopped. Accordingly the length of time the apical end continues to grow will determine the final position of the mature papilla. And furthermore, at this time added evidence has been obtained to show that there is a definite relationship between epidermal-cell length and length of the full-grown hair. The longest cells are either hairless or produce merely papillae, while the shortest cells produce the longest hairs. The

changes produced on the elongating epidermal cells of tomato roots in the various aqueous solutions are extremely pronounced and the results are in accordance with the theory, suggested by Roberts (1916) and substantiated by Cormack (1935), that root hairs are evaginations produced by internal pressure on plastic portions of an unequally hardened cell wall. They also corroborate the further observation (Cormack, 1935) that the hardening is due to the incorporation of calcium into the primary cell wall.

The discovery that different parts of an epidermal cell are growing at different rates at the same time, may serve to explain the reason for the emergence of a hair at or near the apical end. Through the progressive hardening of the walls at the basal end and from the increasing pressure, the still extending portion of the outside wall at the apical end becomes bulged. That the wall is more plastic here is indicated by the wide diameter of the bulge itself and by the ability of the apical end to grow in length after the bulge has pushed out. The length of time the apical end continues to grow will determine the final position of the papilla in the full-grown cell. Under conditions where hardening of the walls takes place progressively but much more rapidly, the apical end begins to harden at the same time that the bulge protrudes. Thus the cells remain short, and the more plastic area of the bulge becomes the incipient root-hair tip. Through the progressive hardening of the outer layer (Cormack, 1935) this softer spot is confined to a narrow area at the growing tip where new wall material is being constantly laid down and this determines the narrow diameter of the growing hair. Thus differential cell-wall growth becomes established in the hair itself and elongation of the epidermal cell continues in a new direction.

SUMMARY

Evidence obtained by measuring the epidermal cell walls of tomato roots indicates that the cells are still growing in the region where the papillae first make their appearance. Additional evidence obtained by the use of various aqueous solutions once again demonstrates that the capacity to form hairs is determined by the chemical and physical changes which take place while the cells are still elongating.

In tomato roots a hair emerges as a wide bulge near the apical end of a cell where the wall is more plastic. Under conditions where the walls harden slowly the more plastic nature of the apical end allows for further longitudinal extension of the cell after the bulge pushes out. As a result the papilla occupies a central position in the full-grown cell. Conditions which speed up calcification of the middle lamellae inhibit further elongation at the apical end, growth becomes localized in the bulge and a long straight hair results.

The epidermal cells of tomato roots are all of the "long-cell type," not well adapted to produce hairs under most conditions in aqueous solutions. It is only

under limited conditions where calcification takes place progressively, but rapidly, that all the cells remain short and produce long hairs.

DEPARTMENT OF BOTANY,
UNIVERSITY OF ALBERTA,
EDMONTON, ALBERTA, CANADA

LITERATURE CITED

- BRUMFIELD, ROBERT T. 1942. Cell growth and division in living root meristems. *Amer. Jour. Bot.* 29:533-543.
- CORMACK, R. G. H. 1935. Investigations on the development of root hairs. *New Phytol.* 34:30-54.
- . 1937. The development of root hairs by *Elodea canadensis*. *New Phytol.* 36:19-25.
- . 1944. The effect of environmental factors on the development of root hairs in *Phleum pratense* and *Sporobolus cryptandrus*. *Amer. Jour. Bot.* 31:443-449.
- FARR, C. H. 1925. Root hair elongation in Knop's solution and in tap water. *Amer. Jour. Bot.* 12:372-383.
- . 1927. Studies on the growth of root hairs in solution. 1. The problem, previous work and procedure. *Amer. Jour. Bot.* 14:446-466.
- . 1928. Studies on the growth of root hairs in solution. 6. Structural responses to the toxic pH and molar concentration of calcium chloride. *Amer. Jour. Bot.* 15:171-178.
- . 1928a. Studies on the growth of root hairs in solution. 8. Structural and intracellular features of collards in calcium nitrate. *Bull. Torrey Bot. Club* 55:529-553.
- . 1928b. Root hairs and growth. *Quart. Rev. Biol.* 3:343-376.
- FARR, WANDA K. 1927. The elongation of root hairs in solutions of single calcium compounds. A comparison of three species, *Brassica oleracea*, *Avena sativa*, *Oryza sativa*. (Abst.) *Amer. Jour. Bot.* 14:627.
- . 1929. The elongation of root hairs of *Brassica oleracea* in solution of primary calcium phosphate. (Abst.) *Amer. Jour. Bot.* 16:852.
- HULBARY, ROBERT L. 1944. The influence of air spaces on the three-dimensional shapes of cells in *Elodea* stems, and a comparison with pith cells of *Ailanthus*. *Amer. Jour. Bot.* 31:561-580.
- JEFFS, R. E. 1925. The elongation of root hairs as affected by light and temperature. *Amer. Jour. Bot.* 12:577-606.
- MCPHERSON, D. C. 1939. Cortical air spaces in the roots of *Zea Mays* L. *New Phytol.* 38:190-202.
- ROBERTS, EDITH A. 1916. The epidermal cells of roots. *Bot. Gaz.* 62:488-506.
- SINNOTT, EDMUND W. 1939. Growth and differentiation in living plant meristems. *Proc. National Acad. Sci. (U.S.A.)* 25:55-58.
- , AND R. BLOCH. 1939. Cell polarity and the differentiation of root hairs. *Proc. National Acad. Sci. (U.S.A.)* 25:248-252.
- , AND ———. 1939a. Changes in intercellular relationships during the growth and differentiation of living plant tissues. *Amer. Jour. Bot.* 26:625-634.
- SNOW, LAETTITA M. 1905. The development of root hairs. *Bot. Gaz.* 40:12-48.

GROWTH FACTOR STUDIES WITH SPIRODELA POLYRRHIZA (L.) SCHLEID.¹

Paul R. Gorham²

CONSIDERABLE CONTROVERSY has attended the question as to whether organic manures, humus, peat, or soil are able to supply small amounts of organic materials which promote the growth of green plants, or whether these substances correct recognized or unrecognized inorganic deficiencies.

Bottomley (1914, 1917a, 1917b, 1919, 1920a, 1920b) was one of the earlier workers to investigate organic growth promoters and their effect on higher plants. He and Mockeridge (1920, 1924a, 1924b) studied the effects produced upon the growth of *Lemna* by adding extracts of bacterized peat, *Azotobacter chroococcum*, *Bacillus radiculicola*, *Saccharomyces*, fresh and well-rotted stable manure, manured soil, and leaf-mold to inorganic nutrient solutions. For a basal medium they used Detmer's³ and later Knop's⁴ formula upon which

Lemna failed to grow normally. They erroneously concluded that "auximones," thought to be in the nature of purine and pyrimidine bases, were essential for the growth of green plants.

Clark (1924) and Saeger (1925) demonstrated that *Lemna* and *Spirodela* maintained vigor and grew normally in a properly constituted inorganic nutrient solution. Ashby (1929b), growing *Lemna minor* upon Clark's⁵ nutrient solution, found that extracts from fresh and from well-rotted manure produced a marked growth increase. The active materials withstood autoclaving, but not ashing. Since stimulated plants had larger cells with more chloroplasts, he attributed the increased growth rate to increased photosynthesis. Olsen (1930) found that bacterized peat extract added to Knop's solution promoted growth of *Lemna polyrrhiza*. This effect could be equalled by substituting ferric citrate for ferric chloride in the medium; therefore, Olsen concluded that humus extract made iron more readily available to the plants. Clark (1930), and Clark and Roller (1931) grew *Lemna major* in KH_2PO_4 0.167, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.167, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 0.5, FeCl_3 few drops.

⁵Concentration in grams per liter: $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ 0.07, KNO_3 0.8, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.25, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.0027.

¹Received for publication March 17, 1945.

²The work reported in this paper was done at the William G. Kerckhoff Laboratories of the California Institute of Technology, Pasadena, California. The author wishes to express his appreciation for the helpful suggestions of Dr. F. W. Went, under whose supervision this investigation was carried out.

³Concentration in grams per liter: KNO_3 2.33, KH_2PO_4 0.5, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, NaCl 0.5, $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ 1.67, FeCl_3 trace.

⁴Concentration in grams per liter: KNO_3 0.167,

sterile and non-sterile culture to test the effect of extracts from soil, alfalfa, and manure. In sterile culture the rate of frond multiplication was unaffected or else depressed by the addition of extracts while in non-sterile culture their addition caused the rate to increase. Without additions growth was better in sterile than in non-sterile culture. Pure cultures of bacteria introduced into sterile inorganic cultures sometimes increased, sometimes decreased growth, or else had no effect. Decreased growth was less pronounced if organic matter was present. Urea and creatinine had no effect in either sterile or non-sterile culture.

In an attempt to clarify further the role that extracts from peat, soil, and manure play in plant nutrition the following investigation was undertaken.

MATERIAL AND METHODS.—Saeger (1930) described a method for obtaining pure cultures of *Spirodela polyrhiza* by treatment of the resting buds with 10 per cent Zonite. About half of the plants which were treated according to a modification of the method of Hopkins (1931) survived and produced new fronds. A plant of only one or two fronds, held gently by means of forceps, was submerged for 45 seconds in 0.1 per cent mercuric chloride, rinsed briefly in sterile water, submerged for 30 seconds in 50 per cent ethyl alcohol, rinsed twice in sterile nutrient, then transferred to sterile nutrient for growth. The clone of *Spirodela polyrhiza* used in all experiments was derived from one of the tested microorganism-free plants and was maintained by subculturing for 2½ years with only occasional contaminations.

Light culture.—Plants were grown in cotton-stoppered Pyrex culture tubes, 175 mm. by 20 mm. Twenty-five milliliters of Hoagland's⁶ nutrient solution, with or without added test material, were pipetted into each tube. Each tube of sterile medium was inoculated with *Spirodela* plants sufficient to give a total of 30 fronds. After allowing one week for adaptation to new medium, a subculture was made by transferring 30 fronds, and the growth of this was measured one to two weeks later. For growth, "inoculated" tubes were placed in special racks inclined 70 degrees from the perpendicular (fig. 1). These were placed upon shelves, adjustable for height, which had 40-watt "White" fluorescent lamps suspended above them. The shelf-bottom from which each lamp was hung served as its reflector. Variations in light intensity were produced by raising or lowering the shelves. At distances of 4 and 16 inches from the source, the light intensity was approximately 300 and 100 foot-candles respectively. Two sets of aluminum-painted shelves with lamps were located equidistant from the aluminum-painted walls of a windowless room which was

temperature-controlled at $25 \pm 1^\circ\text{C}$. Each set consisted of three lamp-shelf units. All six lamps were operated continuously, even when units were not in use, so that the light reflected from the walls remained relatively constant throughout the experiments. At the far end of the room, screened from the "White" fluorescent units, an 80-watt Cooper-Hewitt fluorescent was suspended above a table also to provide a light intensity of approximately 300 foot-candles at the culture surface.

When tested with a Weston Photronic cell and galvanometer, the light intensity along the culture racks was found to lack uniformity and to vary slightly beneath the different lamps. Therefore the tubes were shifted in the racks each day, and the racks were shifted about from unit to unit. This was done in such manner that each culture, over a period of one week, received approximately the same total amount of light energy.

In a study of the effects of aeration, each culture was provided with a capillary which extended almost to the bottom. This was inserted, along with an exhaust tube, through a rubber stopper in the mouth. The outer openings of both inlet and exhaust were loosely packed with cotton (fig. 2) to permit sterile culture. By connecting a number of such cultures with rubber hose it was possible to aerate them at the same rate.

The increment in frond number (final count minus inoculum) and changes in frond size, fresh weight per 100 fronds, and dry weight per 100 fronds, occurring in the subcultures during a stated period, served as criteria of growth.

Frond size was derived from a measurement of the total area of a culture. For this purpose, silhouettes of the plants in each culture, as well as of a series of discs of known area, were obtained by photography and compared by means of a photoelectric photometer.

The photographic apparatus consisted of a modified 35-mm. camera, with a focal length adjusted to eight inches by means of an extension tube. This was mounted to travel at fixed focus above a polished brass container 28 inches long, 1⅜ inches wide, and 1½ inches deep, having a bottom of opal glass (fig. 3). This container had twelve compartments of such dimensions that the focussed image had very nearly the same dimensions as the aperture above the photo-cell of the photometer. The separating walls between the compartments did not extend quite to the bottom of each chamber, so that when the container was filled to the brim with water, the concave meniscus in each chamber was equidistant from the camera lens. Attached to the carriage supporting the camera and moving with it beneath the glass bottom of the container was an illuminating box containing a 7-watt pilot lamp and a 250-watt photoflood lamp.

For use, the inner compartment walls were first polished for high reflectivity, then the container was filled to the brim with water. With a camel's-hair brush, the plants of a single culture were trans-

⁶ Concentration in grams per liter glass-distilled water: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 1.180, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.493, KNO_3 0.506, KH_2PO_4 0.136, ferric tartrate 0.005. Minor elements in milligrams per liter: H_3BO_3 2.86, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ 1.81, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ 0.22, MoO_3 0.07, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 0.08.

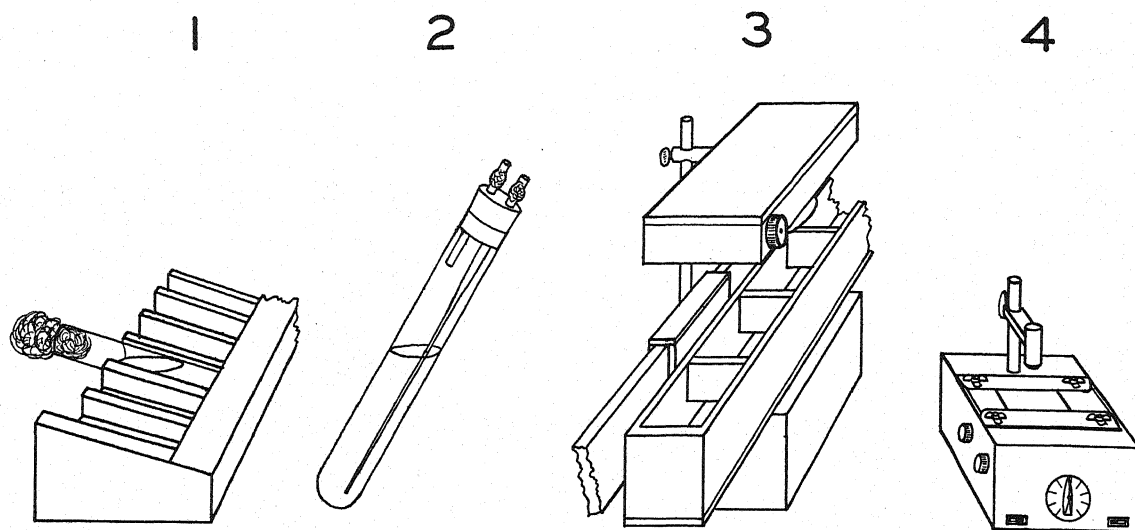


Fig. 1-4.—Fig. 1. Inclined culture rack with tube.—Fig. 2. Aerated culture tube.—Fig. 3. Compartmented container, traveling camera and illuminating box of photographic apparatus.—Fig. 4. Lamp and photoelectric cell assembly for photometering film.

ferred to a single compartment, but where growth was extraordinary, two or sometimes three compartments were used. Two of the twelve compartments were always reserved for varying numbers of standard discs. A check was made to see that all plants floated free and were not overlapping one another. Silhouettes of the contents of each compartment were recorded by photographing with the light from the illuminating box. The plants were then saved on moist blotting paper in a covered pan for weight determinations.

An identification picture was taken at the beginning of each film of 36 exposures. Careful notes were kept, frame by frame, film by film, of the sequence of cultures photographed, and any errors noted so that there might be no confusion as to the culture a particular silhouette represented. Inexpensive positive or sound-recording film of Weston speed rating of about 1.0 was found very satisfactory. Exposure at $f/4.5$, $1/200$ seconds, followed by tank development at 18°C . for 2.5 minutes with Eastman D-8 developer, gave contrast such that the image of a plant was transparent, with no detail, while the background was black and virtually opaque.

The photometer was constructed with a film guide permitting careful orientation of each frame directly over the photo-cell. An adjustable mask was used to correct for slight variations in frame dimensions. The setting of the mask was unchanged while photometering any one film. A 6-volt, 0.25-ampere lamp illuminated the photo-cell. It was operated from a storage battery at 0.25 amperes, carefully maintained by means of variable resistances. This lamp was mounted on a bracket two inches above the photo-cell with a lens in front of the housing to concentrate the light evenly over the photo-cell surface (fig. 4).

The apparatus was used in a darkroom. The deflection of a sensitive galvanometer attached to the photo-cell was used for determining current (and thus area) variations in most of the experiments. In later experiments these current changes were measured potentiometrically so as to overcome the range limitations imposed by the galvanometer scale. On each film, spaced at regular intervals, were silhouettes of 4, 8, 12, 16, 18, and 20 of the floating discs of known area. By means of these it was possible to construct curves for converting photometric readings into terms of area in square millimeters. Development produced variations in density from film to film which necessitated the inclusion of a complete set of reference standards on each film.

Five standard discs were photographed in each of the twelve compartments and twelve determinations of area were made. The five discs, with an area of 135 sq. mm., had a standard deviation of 4.5 sq. mm. Likewise ten discs, with an area of 270 sq. mm., had a standard deviation of 3.2 sq. mm.; while 20 discs, with an area of 540 sq. mm., had a standard deviation of 4.2 sq. mm. Since most of the determinations were in the range of 400 to 500 sq. mm., the measurements were quite precise.

Fresh weight was determined by taking each culture, blotted to remove surface moisture, and rapidly weighing it to the nearest milligram on a balance of the chain-o-matic type. If dry weight was to be determined, the plants of each culture were then placed in a small folded paper packet, dried for 24 hours in a forced-air dryer at 40°C ., and weighed to the nearest 0.1 milligram.

From an experiment in which treatment was replicated ten times, the average value for increment in frond number was 223 ($\sigma = 10.7$), fresh

weight per 100 fronds 79 mg. ($\sigma = 6.7$), and dry weight per 100 fronds 10 mg. ($\sigma = 0.62$).

Dark culture.—Plants were grown in darkness under environmental conditions otherwise comparable to those employed in light. Sucrose was supplied at a concentration of 2.5 per cent. Since short exposure to low intensities of light increased subsequent growth in darkness, sextant green ($>4600\text{\AA} < 6000\text{\AA}$), the least effective in this regard, was chosen to filter the 40-watt Mazda lamp used to illuminate transfer operations. Cultures were transferred at a distance of three feet from the safelight, to one side of the direct rays. In preliminary tests, growth measurements were made upon subcultures which had been inoculated with 30 fronds taken from cultures grown for seven days on test media in darkness. Because new fronds form clumps, *i.e.*, do not absciss readily, when grown on sucrose medium, subculturing by dim green light was difficult. Therefore, an inoculum of 30 fronds was taken from sucrose medium in the light and grown two weeks in darkness to deplete reserves of growth materials; then the whole culture was transferred to test medium, grown two to three weeks, and measured. Increment in frond number and changes in fresh and dry weight per 100 fronds served as criteria of growth.

EXPERIMENTAL.—Light intensity, inorganic nutrition.—In a number of preliminary experiments no organic test materials were added to the basal medium. These tests served to establish the proper size of inoculum and the best time for subculturing. When growth at a distance of 16 inches from "White" and "Daylight" fluorescent lamps was compared, frond number and fresh weight were found to be greater under the "White" type. This was correlated with the difference in luminous output of the two lamps rather than with spectral differences. Cultures were grown under different light intensities and photoperiods. Over the ranges studied the resulting growth was proportional to the total luminous energy received, just as Clark (1925), and Ashby (1929a) found with the related genus *Lemna*. With a light intensity of 300 f.c., it was observed that a considerable number of fronds became impregnated with water and sank to the bottom of the tube. With an intensity of 100 f.c., the percentage of these "water-soaked" plants was greater if the basal medium was diluted, if the concentration of KH_2PO_4 was reduced, if KNO_3 was omitted, or if the cultures were aerated. A potassium deficiency appeared to be responsible for the condition since it virtually disappeared at lower light intensities, and could be corrected at higher light intensities by doubling the concentration of KNO_3 , even in aerated culture.

A study was made of the major element requirements of *Spirodela* by the method of triangulation, in which concentrations of $\text{Ca}(\text{NO}_3)_2$, KH_2PO_4 , and MgSO_4 were varied from 1.0 to 6.0 millimoles, in the presence and absence of KNO_3 (5.0 millimoles). Minor elements were supplied according

to Hoagland's formula. In the series with KH_2PO_4 at 1.0 millimole concentration growth was optimal, regardless of the proportions of the other salts. Two series with KH_2PO_4 at concentrations of 0.5 and 1.0 millimoles showed the higher concentration to be better, while the proportions of the other salts were not critical. Therefore, Hoagland's formula, which has KH_2PO_4 at a concentration of 1.0 millimole, was adopted for the basal medium as being able to produce essentially optimal growth under the defined environmental conditions.

Aeration, carbohydrates.—Frond multiplication, frond size, and frond weight were all increased by aeration (table 1). It made no difference whether

TABLE 1. The effect of aeration on the growth of *Spirodela*. Growth period eleven days, light intensity 300 f.c.

	Non-aerated (mean of 4 replicates)	Aerated (mean of duplicates)
Increment in frond number.....	99	121
Frond size (sq. mm.).....	3.4	4.4
Fresh weight/100 fronds (mg.)..	76	94

air was bubbled through the medium or only wafted across the surface. Treating with 0.5 and 5.0 per cent carbon dioxide-air mixtures produced an increase in growth. When aerated and carbon dioxide-air treated plants were analyzed for combined reducing sugars and sucrose, they were found to contain very nearly the same amounts, which were 100 per cent higher than the sugar content of the unaerated controls.

Hopkins (1931), and Steinberg (1941) have reported that 0.5 per cent glucose added to an otherwise satisfactory inorganic medium greatly promotes frond multiplication of *Lemna*. The latter's data show that glucose also increases the size of the individual fronds.

When glucose and sucrose were tested in concentrations of 0.5 and 1.0 per cent with *Spirodela*, growth stimulation was pronounced. The higher concentration in each case promoted growth to a greater degree. Sucrose was somewhat more effective than glucose.

Five sets of cultures, in duplicate, containing 0, 0.5, 1.0, 2.5, 4.0, and 5.0 per cent sucrose were prepared. Three sets were grown at 100 f.c.: one set unaerated, one set with 4 per cent carbon dioxide-air bubbled through, and one set beneath a bell jar in an atmosphere of 4 per cent carbon dioxide-air. The two remaining sets were grown at 300 f.c.: one set unaerated, the other set with 4 per cent carbon dioxide-air bubbled through. The range of sucrose concentration from 1.0 to 4.0 per cent produced the greatest increase in frond number (fig. 5), but frond size was greatest between 0.5 and 2.5 per cent (fig. 6). The additive effects of carbon dioxide and sucrose treatments upon increment in

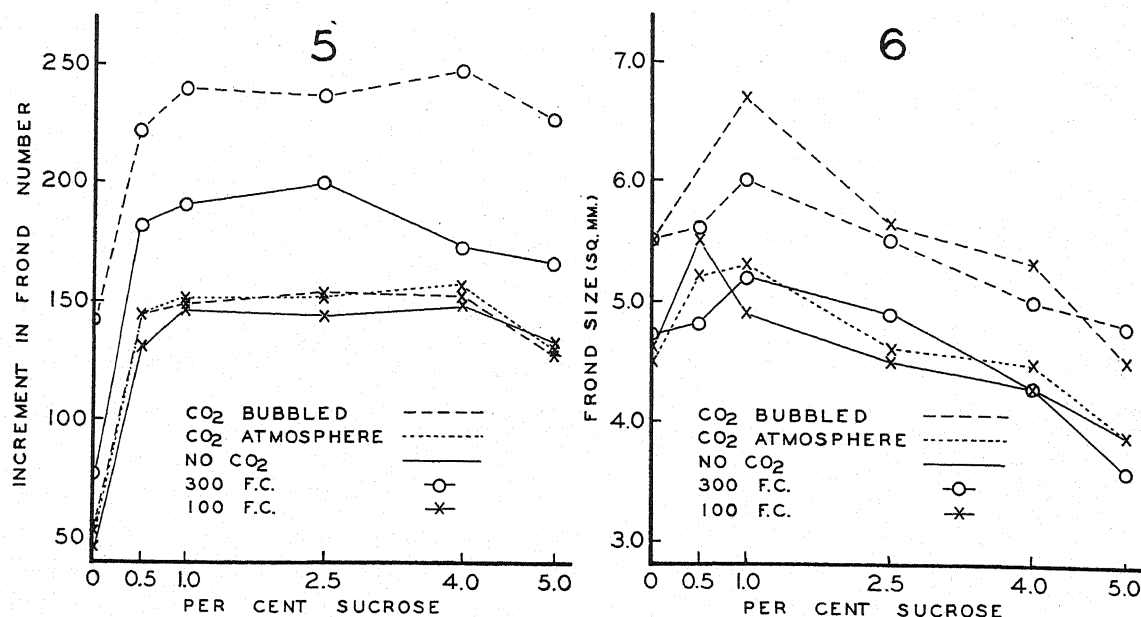


Fig. 5-6. Effect upon frond multiplication (fig. 5) and upon frond size (fig. 6) of treating cultures having varying amounts of sucrose with an atmosphere of 4 per cent CO_2 -air or a stream of 4 per cent CO_2 -air bubbled through the media. Points represent means of duplicates. Growth period 7 days.

frond number were both intensified by increased illumination. Frond size, however, increased in response to both sucrose and carbon dioxide treatments, but not in response to increased illumination. Either an atmosphere enriched with carbon dioxide or a stream of carbon dioxide-air bubbled through the medium produced an increase in frond number. Frond size, however, increased only when carbon dioxide was bubbled through the medium.

Although it was not possible to employ a sucrose supplement in place of light and carbon dioxide relations optimal for photosynthesis, nevertheless, its recognition as a distinct growth factor made its inclusion in the test media of subsequent experiments obligatory.

Peat and dung extracts.—Eighty-seven grams of sphagnum peat were dried, extracted with water at pH 8.5 in a Waring blender, centrifuged, the clear liquid concentrated under reduced pressure, neutralized with hydrochloric acid, and made up to 250 ml. Samples of fresh horse dung and cow dung were dried, 100 grams of each autoclaved in 500 ml. of distilled water, centrifuged, and made up to 500 ml. volume. These extracts were tested in 0 to 5.0 per cent sucrose media by adding 1.0 ml. per culture. Growth was compared at light intensities of 100 and 300 f.c. The higher light intensity produced an added increment in frond number and fresh frond weight for all treatments, but affected frond size very little. The effects were maximal in the range of sucrose concentration from 1.0 to 3.0 per cent. Each of the extracts was tested at the same time over a range of concentration of 0 to 5.0 ml. per culture (representing extract from

0 to 1.75 grams) in 2.5 per cent sucrose medium. The higher light intensity somewhat accentuated the effects produced by the lower concentrations tested. At 300 f.c., all three extracts increased or decreased the rate of frond multiplication, depending upon concentration (fig. 7). Extracts of cow dung and horse dung tended to increase frond size and fresh weight with increasing concentration, but the extract of sphagnum produced marked decreases (fig. 8, 9). A pronounced increase in hydrogen-ion concentration of the sphagnum media, from pH 4.8 to 3.0 after seven days' growth, also distinguished it from the other extracts.

Less concentrated extracts of dried sphagnum, horse dung, and cow dung were prepared with the blender and centrifuge, using 50 grams of each made up to 1.0, 1.5, and 1.5 liters, respectively. When a comparative test over a range of concentrations was made at 300 f.c., in the presence and absence of 2.5 per cent sucrose, the presence of sucrose in the medium was found to be essential for the production of an increase in the rate of frond multiplication, frond size, and fresh weight. At concentrations too dilute to affect the rate of frond multiplication, the extract of horse dung produced fronds that were 20 per cent larger than those of the controls.

One hundred milliliters of each of the less concentrated extracts were evaporated to dryness and the residue ashed, taken up in concentrated hydrochloric acid, and made to 25 ml. volume. Each ash solution was tested in 2.5 per cent sucrose medium using 0, 0.1, 1.0, and 5.0 ml. per 25 ml. This represented the ashed extract from 0 to 1.0 grams of dry

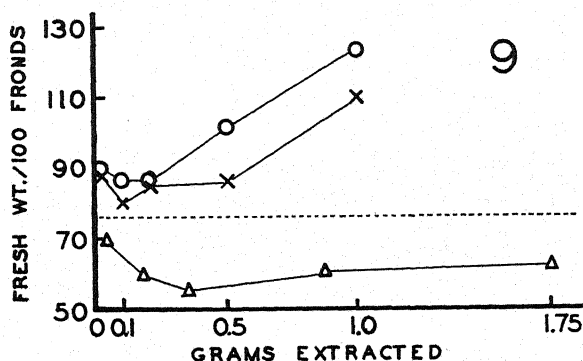
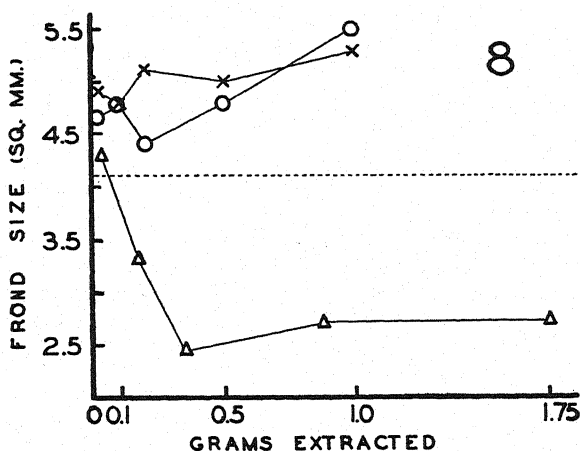
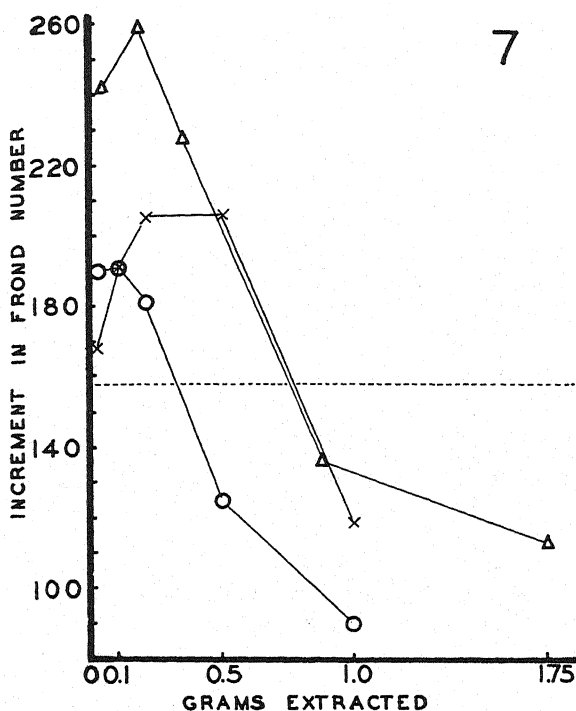


Fig. 7-9. Effects upon increment in frond number (fig. 7), frond size (fig. 8), and fresh weight (fig. 9) of aqueous extracts of dried sphagnum peat (triangles), horse

material. The pH of the prepared media was adjusted to 5.2 with a few drops of dilute sodium hydroxide. The light intensity was 300 f.c. The ashed extracts at low concentration promoted frond multiplication but little, and tended to inhibit it at higher concentration (fig. 10). Frond fresh weight

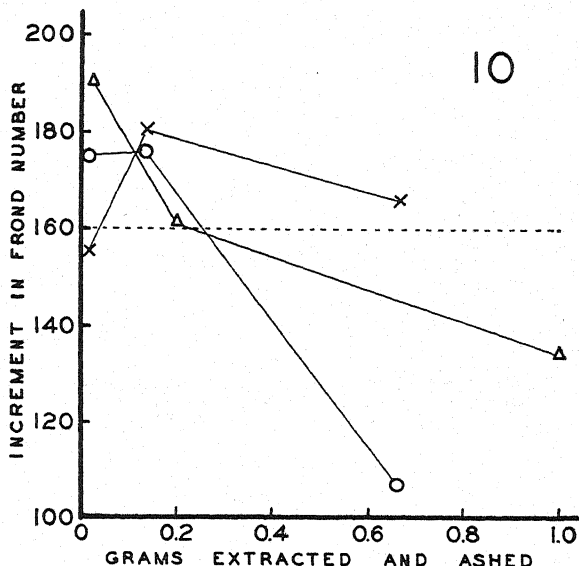


Fig. 10. Effect upon increment in frond number of ash from extracts of dried sphagnum peat (triangles), horse dung (X's), and cow dung (circles). Dotted line at 160 represents mean of 6 untreated replicates, other points represent means of duplicates. Light intensity 300 f.c., growth period 7 days.

no longer differed from that of the controls. In the 5.0 and 1.0 ml. treatments, and to a somewhat lesser extent in the 0.1 ml. treatments, the plants were exceptionally deep green in color with a glossy appearance, just as when treated with whole extracts.

Fifteen grams of dried cow dung were extracted with peroxide-free ether, another 15 grams with methanol, and another 20 grams with acetone, using Soxhlet extractors for 40 hours. After storage at 5°C. for several days, the solvents were distilled off under vacuum, and the residues dissolved as much as possible in water. Insoluble material was removed by centrifugation, and each of the three extracts made up to 250 ml. Meanwhile, the solid residues after solvent extraction, and also 20 grams of unextracted cow dung, were each extracted with water for 15 minutes in a Waring blender, centrifuged, and made to 500 ml. volume. The three solvent extracts, the water extract, and the three residue extracts were tested immediately in concen-

dung (X's), and cow dung (circles). Dotted line represents control. Light intensity 300 f.c., growth period 7 days.

trations of 0, 0.1, 1.0, 5.0, and 10.0 ml.⁷ per 25 ml. of 2.5 per cent sucrose medium. The solvent extracts proved to be quite acid; therefore, the pH of the media was adjusted to 5.3 with dilute sodium hydroxide. The light intensity was 300 f.c. In figure 11 the values for increment in frond number have been plotted against the amount of dried cow dung, or residue after solvent extraction, which must be extracted by each method to produce the indicated effect. Solvent extraction (solid lines) removed an inhibitor for the most part, with the exception of ethyl ether which removed a promoter as well. The residues (broken lines) from the acetone and ether extractions contained growth-promoting material. The residue from the acetone extraction, in particular, appeared to be largely freed from inhibitor and to have lost only a small amount of promoter substance by the extraction. Frond size and fresh weight were little affected by the treatments.

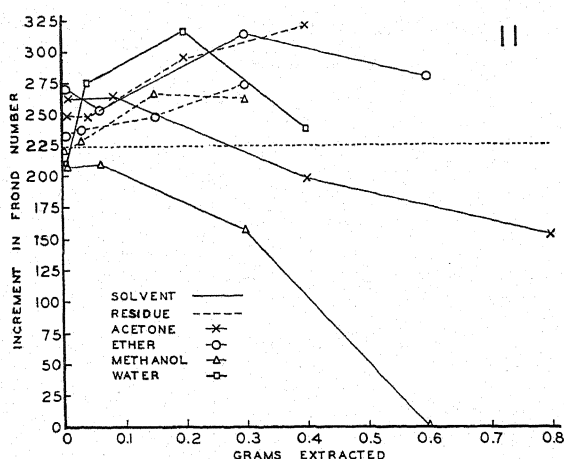


Fig. 11. Effects of different solvent and residue extracts of dried cow dung upon increment in frond number. Dotted line at 223 represents mean of 10 untreated replicates. Other points represent means of duplicates. Light intensity 300 f.c., growth period 8 days.

In this experiment the residue extracts produced deep green, glossy plants, while the solvent extracts produced plants with the same yellowish-green color as the controls. With concentrations of aqueous extract high enough to inhibit the rate of frond multiplication, the tips and margins of the fronds curled up, making an angle of as much as 30 degrees with the plane of the solution surface. The curling phenomenon was not apparent in the cultures treated with ether extract, but the highest concentration of extract from the ether residue

⁷ Ten milliliters represents the extract from the following amounts of dried cow dung or residue after solvent extraction:

	Solvent extracts	Residue extracts
Acetone	0.8 grams	0.4 grams
Ether	0.6	0.3
Methanol	0.6	0.3
Water	0.4	..

caused the frond margins to curl as much as 30 degrees. The methanol extract caused a pronounced curling while the residue after methanol extraction caused none. Curling was apparent to a lesser degree both in treatments with acetone extract and acetone residue extract.

In a 2.5 per cent sucrose culture a chance bacterial contaminant, which appeared to be a short rod type under microscopic examination, increased frond number by 40 per cent, frond size by 10 per cent, but decreased fresh weight by 6 per cent and dry weight by 27 per cent. The plants were larger, greener, more delicate-looking than those in uncontaminated cultures.

Growth in darkness.—In a preliminary test, subculturing by the aid of a red safelight, the increment in frond number after 18 days was recorded. Controls without sucrose produced no new fronds, 0.1 per cent sucrose 34 fronds, 1.0 per cent sucrose 128 fronds, while a supplement of growth factors⁸ produced fewer, smaller fronds than the respective sugar concentrations alone. When transferred to sucrose medium the control plants multiplied, seemingly unharmed by more than three weeks of comparative dormancy. All cultures were transferred to 2.5 per cent sucrose medium after counting. After 18 more days, or a total of 43 days in darkness, only those cultures which had received the growth factor supplement were still multiplying, the others having virtually ceased.

Plants which had been growing in darkness on sucrose medium for a month provided an inoculum of ten fronds per culture with which to test various organic growth-promoting substances. Periodic counts of frond number were made up to 53 days. Concentrations deemed likely to be effective, together with dilutions of one-tenth and one-hundredth were tested. The rate of frond multiplication was somewhat improved by glycine, vitamin B₁, vitamin B₂, calcium pantothenate, and adenine; while nicotinic acid, indoleacetic acid, yeast extract, sphagnum extract, and coconut milk scarcely affected it at all. Ammonium sulfate, and aqueous extracts of cow dung and of horse dung significantly retarded frond multiplication.

Cultures depleted of growth-promoting reserves were transferred to test media in a study of the effect of ten growth substances in different combinations. Concentrations in milligrams per liter were: vitamin B₁ 0.1, vitamin B₂ 0.1, vitamin B₆ 0.1, *p*-aminobenzoic acid 0.1, calcium pantothenate 0.1, adenine 0.1, inositol -0.0, asparagine 100.0, glycine 3.0, and biotin 0.0004. Determinations of the over-all increment in frond number and dry weight were made after three weeks' growth on the test media. Table 2 contains the data for increase in frond number. All cultures containing asparagine had nearly twice as many fronds as had the con-

⁸ Concentrations in milligrams per liter: glycine 3.0, thiamin 0.15, ascorbic acid 20.0, nicotinic acid 1.0, vitamin B₆ 0.2, adenine 0.2, succinic acid 25.0, pantothenic acid 0.5.

TABLE 2. *Five weeks' (two without treatment) increment in frond number in darkness, in response to various combinations of ten growth substances. Means exclude those with asparagine or p-aminobenzoic acid. Control (mean of 5 replicates) = 79.*

	B ₁	B ₂	B ₆	p-amino- benzoic acid	Inosi- tol	Calcium panto- thenate	Biotin	Aspara- gine	Glycine	Adenine	Mean
B ₁ B ₂	93	70	105	100	109	156	72	92	95
B ₂ B ₆	93	58	90	84	64	146	84	73	81
B ₆ p-aminobenzoic acid.	41	58	57	64	52	164	60	80	...
p-aminobenzoic inositol..	67	73	57	73	38	149	70	74	...
Inositol calcium panto- thenate	85	68	102	73	72	163	83	79	82
Calcium pantothenate biotin	96	87	85	59	72	158	83	98	86
Biotin asparagine	216	140	152	169	150	158	169	166	...
Asparagine glycine	181	180	170	162	175	195	169	137	...
Glycine adenine	75	126	88	66	72	108	73	137	90
Adenine B ₁	92	100	45	77	86	95	183	75	...	88
Mean	87	93	94	...	83	94	83	...	79	85	...

trols. Total dry weight was likewise doubled. All cultures containing *p*-aminobenzoic acid (except those with asparagine) produced fewer fronds than the controls. The other compounds produced no well-defined effects.

Interaction effects were tested further with asparagine, calcium pantothenate, vitamins B₁, B₂, and B₆, indoleacetic acid, and biotin, using two concentrations of each substance. Calcium pantothenate (1.0, 4.0 mg. per l.), the three B vitamins (1.0, 4.0 mg. per l., each constituent), and indoleacetic acid (0.2, 2.0 mg. per l.) showed no effects. Biotin at 0.0004 mg. per l. was slightly inhibitory, and at 0.002 mg. per l., very inhibitory, largely overcoming the effects of asparagine. Asparagine at 100, 400, and 1000 mg. per l. produced a stimulation of frond production which was proportional to concentration (table 3). With the higher concentrations of asparagine, new fronds, as they were produced, became progressively smaller and less vigorous in appearance. Succinic and aspartic acids were also tested, since they are believed to occupy key positions in the scheme of plant nitrogen metabolism (Wilson, 1940). Both concentrations of succinic acid and the lower concentration of aspartic acid inhibited frond production (table 3). Aspartic acid at a concentration of 400 mg. per l. significantly increased the rate of frond multiplication, but the fronds were decidedly abnormal in appearance.

Sixteen amino acids, each in concentrations of 10, 100, and 1000 mg. per l., were tested in groups in an endeavor to find a compound that might replace asparagine. Glutamic and glutaric acids were also tested. No group produced an effect comparable to that of asparagine. Only the combination of cystine and methionine in the lowest concentration significantly increased frond number (table 3).

A test was begun to determine how long cultures could be maintained with successive transfers in darkness on medium to which sucrose and aspara-

gine had been added. Since the optimal asparagine concentration was not defined, a series containing 0, 0.4, 1.0, 2.0, and 3.0 gm. per l. was prepared. Because of the subculturing by dim green light that was in prospect, the method was altered to provide an initial inoculum as little clumped as possible. Therefore, plants were taken directly from non-sucrose medium in the light to inoculate the test media, and then were placed in darkness. The test was discontinued after three weeks. In that time the plants had produced only one or two normal fronds, followed by a number of very tiny fronds which separated from the parent plants and became necrotic. Since the controls multiplied very slowly

TABLE 3. *Final frond counts after treatments in darkness for the periods indicated.*

Expt.	Initial no. fronds (ave.)	Test period (days)	Treatment	Conc. (mg./l.)	Final no. fronds
1	75	14	Asparagine	100	201
				400	367
				1000	444
			Aspartic acid	100	73
				400	145
			Succinic acid	100	72
				400	77
			Control		97
2	205	17	Asparagine	10	406
				20	413
				500	561
				1500	642
			Glutamic acid	10	401
				100	403
			Glutaric acid	100	288
			Cystine + Methionine	10 each	488
				100 each	376
			Control	1000 each	280
					373

but appeared normal, this behavior was not traceable to an error made in the preparation of the basal medium. A sample of asparagine of high purity tested (1.0 gm. per l.) at the same time produced the same results.

DISCUSSION AND CONCLUSIONS.—Despite the degree of environmental control that is possible in the culture of *Spirodela polyrrhiza*, the interpretation of results is complicated by interaction of factors and the lack of a precise definition of growth. Even though increase in frond number, frond size, and frond weight are measurable and represent certain aspects of growth, each aspect may vary independently of, or in relation to each of the other two, depending upon the nature of the treatment.

Since enriching an atmosphere with carbon dioxide is without effect upon frond size, it appears to be the better oxygen supply rather than carbon dioxide supply that produces larger fronds when cultures are aerated. Supplements of carbon dioxide and illumination to provide additional photosynthetic products fail to nullify the growth-promoting effects of sucrose. Instead, its expression is heightened, suggesting that an interdependence between the level of sucrose and the level of other products arising from photosynthesis plays an important part in determining the extent to which a culture can grow.

Aqueous extracts of sphagnum peat, cow dung, and horse dung, when ashed, affect growth but little, although they produce in the plants the same dark green color as do whole extracts. This casts doubt upon the suggestion of Ashby (1929b) that manure extracts improve growth by increasing the number of chloroplasts, thus increasing the rate of photosynthesis.

Organic solvent extraction of cow dung shows that it contains growth inhibitors as well as promoters, both of which affect the rate of frond multiplication. The promoter fraction is insoluble in acetone and methanol, but soluble in ether; while the inhibitor fraction is soluble in acetone and methanol, and insoluble in ether. Acetone extraction followed by ether extraction should separate the promoter fraction for further study. A fraction which possibly is the same as the inhibitor fraction, since it is extracted along with it, causes the frond margins to curl up. Auxins cannot be responsible since they are ether-soluble, and with *Lemna* produce epinastic responses (Gorham, 1941).

The observations of Clark (1930), and Clark and Roller (1931) on the effects of adding extracts of soil, alfalfa, and manure, or pure cultures of bacteria to sterile inorganic cultures of *Lemna major* do not seem in complete agreement with the observations made here. However, extract of horse dung tested in the absence of sucrose produced just the effect these workers describe, namely, little or no growth promotion or else growth inhibition; whereas, in the presence of sucrose, extract of horse dung caused a slight increase in frond number and a significant increase in frond size. The

chance bacterial contaminant which increased growth of *Spirodela* in sucrose culture can be assumed to have released growth-promoting substances to the medium. Some of the inconsistencies reported by these workers in their tests of pure cultures of bacteria might have been remedied by the addition of a carbohydrate source to the inorganic medium in order to accelerate bacterial growth and, indirectly, the production of promoter substances.

The dark-culture experiments reveal that plants that have been grown in the light on sucrose medium are capable of limited frond multiplication and development on sucrose medium when placed in the dark. Without sucrose they remain in a static condition, but are capable of resuming growth in the dark several weeks later when transferred to sucrose medium. Supplements of growth factors may prolong the duration of frond multiplication by decreasing the rate. The previous treatment of the inoculum in light is of critical importance for the full expression of the ability of asparagine to increase the rate of frond multiplication. Asparagine and sucrose are, therefore, inadequate for sustained growth in darkness, since substances produced by the plants in light and accumulated to an appreciable degree only when sucrose is present in the medium, very soon limit growth in the dark. Reference to figures 5 and 6 is of interest in this connection, since these show that an increase in frond number and frond size occurs in the light when plants are grown on sucrose medium. The fact that asparagine so accelerates meristematic activity that successive fronds become smaller and ultimately necrotic, suggests that attention should be directed towards the discovery of factors influencing frond development.

SUMMARY

A method for microorganism-free culture of *Spirodela polyrrhiza* (L.) Schleid. under controlled conditions of light and temperature is described. A procedure for photometrically determining frond area is also described.

Frond multiplication rate increases in response to increased illumination, supplemental carbon dioxide, or addition of sucrose. Forced aeration with carbon dioxide promotes frond multiplication even when sucrose concentration is optimal, while increased illumination heightens the effects of both treatments. Frond size increases with the addition of sucrose or improvement of the oxygen supply, but is not affected by supplemental carbon dioxide or increased illumination.

With a light intensity of 300 f.c. and at suitable concentration, an aqueous extract of sphagnum peat greatly increases the rate of frond multiplication and decreases frond size and fresh weight in sucrose and, to a lesser extent, in non-sucrose culture. Under the same conditions, aqueous extracts of cow dung or horse dung increase the rate of frond multiplication, frond size, and frond fresh weight in

sucrose culture only. The three extracts when ashed have little growth-promoting activity. Dried cow dung contains a fraction that promotes frond multiplication, which is insoluble in acetone and methanol, but soluble in ether. It also contains an inhibitor fraction which is soluble in acetone and methanol, but insoluble in ether. As evidenced by one instance, certain bacterial contaminants may greatly promote the growth of *Spirodela* in sucrose culture.

Plants grown on sucrose medium in the light are capable of limited frond multiplication and development when transferred to sucrose medium in the dark. Plants transferred to medium without sucrose

remain in a static condition, but resume growth in darkness when replaced on sucrose medium. Frond multiplication increases proportionally with added asparagine. This effect depends for full expression upon the previous treatment of the inoculum in light, since substances limiting growth in darkness accumulate to an appreciable degree only in sucrose culture. Tests of 16 amino acids and numerous growth-promoting substances showed that they are unable to replace asparagine in its effect upon meristematic activity.

DIVISION OF APPLIED BIOLOGY,
NATIONAL RESEARCH COUNCIL,
OTTAWA, CANADA

LITERATURE CITED

- ASHBY, E. 1929a. The interaction of factors in the growth of *Lemna*. III. The interrelationship of duration and intensity of light. *Ann. Bot.* 43:333-354.
- . 1929b. The interaction of factors in the growth of *Lemna*. IV. The influence of minute quantities of organic matter upon growth and reproduction. *Ann. Bot.* 43:805-816.
- BOTTOMLEY, W. B. 1914. Some accessory factors in plant growth and nutrition. *Proc. Roy. Soc. London B* 88:237-247.
- . 1917a. Some effects of organic growth-promoting substances (auximones) on the growth of *Lemna minor* in mineral culture solutions. *Proc. Roy. Soc. London B* 89:481-507.
- . 1917b. The isolation from peat of certain nucleic acid derivatives. *Proc. Roy. Soc. London B* 90:39-44.
- . 1919. The effect of nitrogen-fixing organisms and nucleic acid derivatives on plant growth. *Proc. Roy. Soc. London B* 91:83-95.
- . 1920a. The growth of *Lemna* plants in mineral solutions and in their natural medium. *Ann. Bot.* 34:345-352.
- . 1920b. The effect of organic matter on the growth of various water plants in culture solution. *Ann. Bot.* 34:353-367.
- CLARK, N. A. 1924. Soil organic matter and growth promoting accessory substances. *Jour. Indust. Eng. Chem.* 16:249-250.
- . 1925. The rate of reproduction of *Lemna major* as a function of intensity and duration of light. *Jour. Phys. Chem.* 29:935-941.
- . 1930. "Auximones" and the stimulation of *Lemna* by organic matter. *Science* 71:268-269.
- , AND E. M. ROLLER. 1931. The stimulation of *Lemna major* by organic matter under sterile and non-sterile conditions. *Soil Sci.* 31:299-308.
- GORHAM, P. R. 1941. Measurement of the response of *Lemna* to growth promoting substances. *Amer. Jour. Bot.* 28:98-101.
- HOPKINS, E. F. 1931. Manganese and the growth of *Lemna minor*. *Science* 74:551-552.
- MOCKERIDGE, F. A. 1920. The occurrence and nature of the plant growth-promoting substances in various organic manurial composts. *Biochem. Jour.* 14:432-450.
- . 1924a. The occurrence of nucleic acid derivatives in nitrogen-fixing bacteria. *Biochem. Jour.* 18:550-554.
- . 1924b. The formation of plant growth-promoting substances by micro-organisms. *Ann. Bot.* 38:723-734.
- OLSEN, C. 1930. On the influence of humus substances on the growth of green plants in water culture. *Compt.-rend. des Trav. Lab. Carlsberg* 18:1-16.
- SAEGER, A. 1925. The growth of duckweeds in mineral nutrient solutions with and without organic extracts. *Jour. Gen. Physiol.* 7:517-526.
- . 1930. A method of obtaining pure cultures of *Spirodela polyrrhiza*. *Bull. Torrey Bot. Club* 57:117-122.
- STEINBERG, R. A. 1941. Use of *Lemna* for nutrition studies on green plants. *Jour. Agric. Res.* 62:423-430.
- WILSON, P. W. 1940. The biochemistry of symbiotic nitrogen fixation. The University of Wisconsin Press, Madison.

PRODUCTION OF VARIABLE ANEUPLOID NUMBERS OF CHROMOSOMES WITHIN THE ROOT TIPS OF PAPHIOPEDILUM WARDII¹

Robert E. Duncan

THE CHROMOSOME number has been found to differ from cell to cell within the root tips of *Paphiopedilum Wardii* Summerhayes, one of the mottled-leaved Burmese lady slipper orchids. Lack of constancy as regards somatic chromosome number within an individual is not unknown in plants. An occasional cell, for example, or a number of scattered cells, or a group of associated cells within a root may be tetraploid or octaploid. Langlet's (1927) term "polysomaty" is commonly applied in these cases. There are, however, species or varieties in which the somatic complement of chromosomes fluctuates in the vicinity of the diploid number with no evidence of duplication of the entire set. Apparently these variations have diverse histories and are to be explained differently. The chromosome or chromosomes contributing to variability may be supernumerary to the normal complement and much smaller than its members. The B chromosomes of maize are an example (Kuwada, 1919; Fisk, 1927; Longley, 1927) as are the centric fragments surviving from the last previous meioses referred to by Darlington (1937). The variability, on the other hand, may involve replication of members of the complement. There has been little observation on mitoses leading to varying numbers other than through polysomaty.

Since the mechanism by which the change in number is brought about can be followed with considerable ease in *P. Wardii*, a brief investigation was made. The process involves only certain members of the chromosome set and leads to cells polysomic, in Blakeslee's terminology, for those members of the complement. The causal mechanism bears close resemblance to that of polysomaty. These facts lead to the adoption of the word "aneusomaty"² as a name for the phenomenon.

Root tips were fixed in Randolph's modification of Navashin's fluid, imbedded in paraffin, sectioned, and stained with crystal violet-iodine. Counts of chromosomes were made in serial sections from the root tip through the embryonic region. Although the plants supplying the root tips came from two different sources, they may be members of a single clone since the species is rare in cultivation and is propagated vegetatively.

The idiogram (fig. 6) shows the complement to contain seven pairs of atelomitic chromosomes of graduated lengths, two shorter pairs having about the same length. Each member of one pair of chromosomes has a secondary constriction which delimits a region similar in size to the smallest chromosomal type of the complement. The remainder of the chromosomes are rod-shaped; they vary

from 27 to 31 in number according to the present observations. Their lengths are graduated so that the longer and shorter rod-shaped chromosomes can be distinguished easily. Some of the types of intermediate length have morphological peculiarities which are sufficient to distinguish them but this group is generally difficult to assort. The rod-shaped chromosomes are not all strictly telomitic; the locations of their centric regions vary from apparently terminal to sufficiently subterminal so that an extremely short second arm is visible.

The somatic number as determined in equatorial plates ranges from 41 to 45 (fig. 3 and 2, respectively). The shortest type of chromosome (no. 20) is almost spherical; it possesses two horn-like projections, one to a chromatid, at the end anterior in polar congression. Because of its shape and the fact that it is from one-half to two-thirds the length of the next shortest chromosome, it is recognized easily. Two to six (fig. 1 and 5) chromosomes of this type are present in various plates; they may occur in several kinds of associations lying on the periphery of the spindle or they may be scattered throughout the plate. The latter condition is more frequent.

The total number of chromosomes, omitting those of type 20, varies from 38 to 40 (fig. 4 and 1). Two additional types of chromosomes have different frequencies of occurrence in equatorial plates. One of these (no. 19) likewise belongs to the class of smaller chromosomes. It has a swollen mid-region and tapers toward each end, the end anterior in polar migration sometimes being stretched. It is almost as easily distinguishable as the shortest chromosomal type. Two, three, or four (fig. 4, 2, and 1) may be present in somatic plates. The other variant (no. 10) is one of the medium-sized chromosomal types, being roughly three times as long as type 20. Like chromosomes of type 20 and 19, it has a single projection or two small projections, depending upon the view and state of division, at its forward end. Two or three of these (fig. 5 and 3) may be present in an equatorial plate.

Table 1 presents an analysis of the equatorial plates shown in figures 1-5.

The variable number of occurrences of three chromosomal types allows similar total number of chromosomes to be reached by different means. The data in table 1 dealing with figures 2 and 5 demonstrate this point.

Chromosomes of type 20 are the most variable in numbers. So frequent are misdivisions of chromosomes of this type that some stages in their progress have been observed. The apparent explanation is that the two daughter chromatids of a type 20 chromosome may dissociate at the end opposite the centric region before or during the early stages

¹ Received for publication March 19, 1945.

² The author wishes to express his appreciation for Prof. C. E. Allen's advice in selecting an appropriate name.

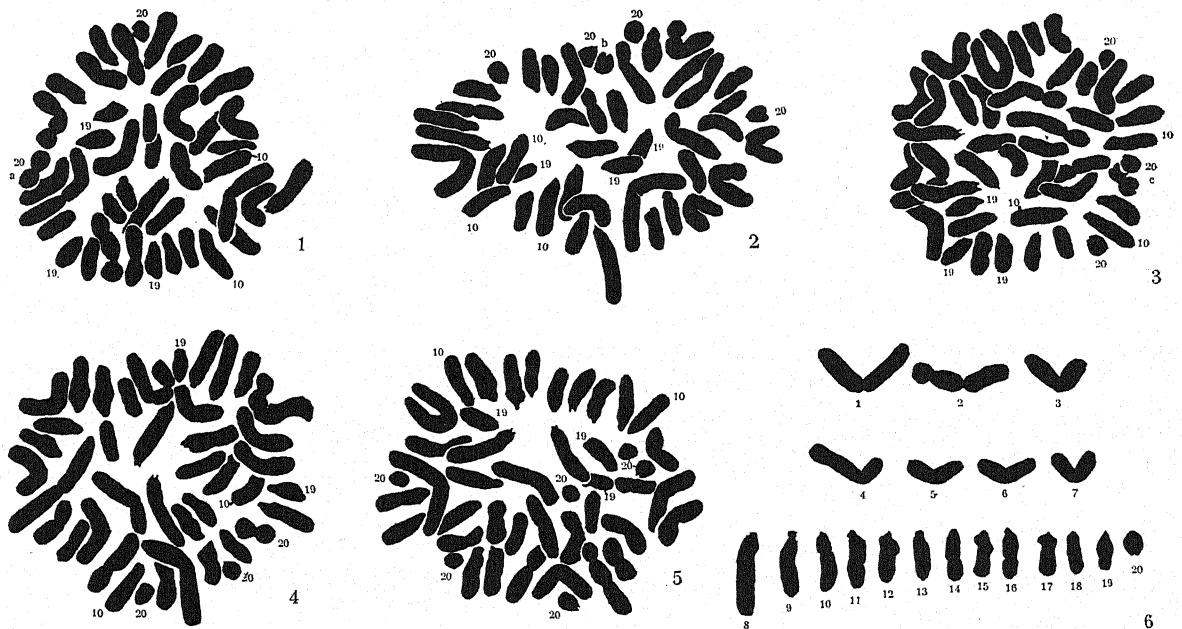


Fig. 1-5. Equatorial plates from root tips of *Paphiopedilum Wardii*. Chromosome types 10, 19, and 20 are marked. Fig. 6. Idiogram of chromosome types, arranged according to centric region and size, present in *P. Wardii*. All figures $\times 1100$.

of nuclear division but remain associated at their centric regions for varying periods of time. The dumb-bell-shaped group labelled 20a in figure 1 or 4 may be interpreted as two chromatids of the same chromosome lying on the equatorial plate and entirely separated but for the centric region. The structure of such a group perhaps can be likened to that of an isochromosome.

The group of two chromosomes of type 20 labelled 20b in figure 2 are putative daughter chromosomes derived from the separation of the two chromatids of a dumb-bell-shaped structure. One of these chromosomes lies at a slightly higher focus than the other. No difference in diameter was detected between divided and undivided chromosomes. The sister chromosomes are themselves double and apparently capable of division in the impending anaphase. Daughter chromatids of all chromosomes separate at about the same time except for those of type 20 which occasionally precede the others in polar congression.

The group labelled 20c in figure 3 illustrates an association of a dumb-bell-shaped structure and a chromosome of type 20. This configuration may have arisen from a misdivision of a chromosome

being followed by similar behavior of one of the daughter chromosomes. The two bodies lie at slightly different levels. Generally chromosomes of type 20 are dispersed throughout an equatorial plate and do not give the appearance of somatic pairing.

Misdivisions of suspects of the other two classes have not been observed. Two replicates of chromosomes of types 10 and 19 occasionally lie side by side and at slightly different foci so that they convey the impression of somatic pairing.

Because of their rod shape in anaphase and their clearly marked terminal regions of spindle fiber attachment, the chromosomes concerned in aneusomaty seem to be telomitic or, if not, the second arm is so short as to be undetected. It would be natural to attribute the misdivisions to the fact that telomitic chromosomes are not stable and lend themselves to a variety of abnormalities (Rhoades, 1940). Several other chromosome pairs of the complement of *P. Wardii* are apparently as definitely telomitic as the three concerned in aneusomaty. Perhaps the relative length of the chromosome as well as the position of the centromere is important in producing aneusomaty. The shortest chromosomal type is replicated most frequently; another

TABLE 1. Frequency of occurrence of chromosomal types in figures 1-5.

	No. 20	No. 19	No. 10	All others	Total
Figure 1	2	4	2	34	42
Figure 2	5	3	3	34	45
Figure 3	4	3	3	34	44
Figure 4	3	2	2	34	41
Figure 5	6	3	2	34	45

relatively short one is also duplicated. The third type is of medium length. If a chromosome concerned in aneusomaty possesses insufficient relational coiling to hold the daughter chromatids together, or if it is excessively contracted, then the terminal position of the centric region may be a contributing factor to the premature doubling. Chromosomes of types 19 and 20 seem greatly contracted. It is noteworthy that no chromosome of the seven atelomitic types occurs more than twice in any of the plates.

Upcott (1939) listed excessive contraction, premature lapse of attraction between sister chromatids, failure in synchronization of dividing centromeres, and hollow spindles as irregularities in mitoses in pollen grains. Such abnormalities of mitosis do not, of themselves, seem capable of bringing about increase in chromosome number except through some unusual segregation of sister chromosomes in which one daughter nucleus gains at the expense of its sister nucleus. Aneusomaty, instead, involves the abnormality of the process which allows sufficient increase of structural material of certain chromosomes so that these may undergo successive divisions while the others are experiencing only one.

Aneusomaty closely resembles polysomaty in several respects. The cells involved are scattered, mostly lying in the cortex or perilem. Such increase in chromosome number as occurs is toward the base of the root. The misdivisions become conspicuous on the equatorial plate, the original chromatids separating first distally and finally at the centric region. The sister chromosomes so formed lie side by side on the plate so that they give the effect of a very close somatic pairing. During the ensuing anaphases each of these daughter chromosomes divides normally so that an increase in number is assured. Duplication of chromatic material must have occurred in the intervening phases of the nuclear cycle. In this respect the work of Berger (1938) on *Culex*, Gentcheff and Gustafsson (1939) on *Spinacia*, and Ervin (1941) on *Cucumis* may be in point. The chief difference between polysomaty as described by Langlet (1927) and aneusomaty in *P. Wardii* is that here only part of the complement is concerned. Cases of polysomaty have been observed in three other species of *Paphiopedilum* (unpublished data).

Earlier explanations of variable numbers of chromosomes in somatic tissue have been numerous. A brief review will be made to relate aneusomaty to these ideas as far as possible. Gates (1912) suggested that chromatids may separate prematurely on the equatorial plate in *Oenothera*. The daughter chromosomes are assorted normally in the ensuing anaphases and the complement is not affected. Montgomery (1901) observed prematurely separated chromatids in animal cells.

Stomps (1916) suggested that $2n - 1$ and $2n + 1$ tissues and cells in certain hybrid *Oenothera* result from the failure of a pair of daughter chromatids to separate, the variant numbers being main-

tained in subsequent divisions. The explanation was used later for the origin of $2n + 1$ branches in *Datura*. This type of variation in somatic number has no apparent relationship to aneusomaty in which cells deficient for some member or members of the chromosome complement do not occur.

Hance (1918) considered that the shorter chromosomes present in cells of *Oenothera* with higher numbers indicate that fragmentation of normal chromosomes accounts for the increase. Kuwada (1919) thought that the small supernumerary chromosomes in maize result from fragmentation of a compound chromosome and that variable numbers arise from variable fragmentation. Winkler (1915) suggested that extra chromosomes in tomato could have arisen through either transverse or longitudinal fragmentation. Separation of the dumb-bell-shaped structure into two chromosomes in *Paphiopedilum Wardii* resembles a transverse break, but actually is a separation of two chromatids and involves no breakage.

Supernumerary "fragments" derived from meiotic irregularities vary in number through gradual loss or increase. Darlington (1937) indicated that the centromeres of these fragments may not be synchronized with the movements of those of members of the complement. As a result both daughter chromosomal fragments may be included within a single nucleus, its sister nucleus suffering a loss in consequence. Chromosomes of types 20, 19, and 10 generally divide at the same time as other members of the complement although one of type 20 may lie slightly nearer the pole than other chromosomes in anaphase figures. The exception to this synchronization is the extra division which occurs on the equatorial plate. The decision as to whether or not chromosomes of types 10, 19, and 20 are fragments could not be made in the material used. Chromosomes of the former two types are large to be considered fragments. In any event the method by which chromosomes of type 20 increase in number per nucleus is distinct from that by which fragments increase in number as described by Darlington.

Aneusomaty as herein reported results in the trisomic to the hexasomic condition for different chromosomal types. The increase is, perhaps, genetically harmless because the chromosomes involved may be inert; otherwise the genetic balance might be influenced unfavorably. Possibly the increase in a few scattered cells might not be harmful.

Little can be said of the role that aneusomaty may play in giving rise to new genetic lines or chromosome races through the usual sexual processes until it is determined whether spore mother cells may become involved and, if so, whether the supernumerary chromosomes are conveyed to functional gametes. Several species of *Paphiopedilum* in the section of the genus to which *P. Wardii* belongs have been observed to propagate themselves vegetatively through buds arising from the embryonic region of the root (unpublished data). Plants of

such origin might include some polysomatic cells in their makeup. This suggests that aneusomaty might be a tool in the production of clonal lines with new aneuploid chromosome complements.

SUMMARY

Various aneuploid complements of chromosomes are present in isolated cells of the root tips of *Paphiopedilum Wardii*. Of the twenty types of chromosomes making up the idiogram, three types may be present in an equatorial plate in numbers

ranging from three to six. This replication at trisomic to hexasomic level occurs through a process which closely resembles that responsible for polysomaty except that not all chromosomes are replicated. The name aneusomaty is suggested for this phenomenon.

THE CHARLES E. ALLEN LABORATORY OF PLANT CYTOLOGY,
DEPARTMENT OF BOTANY,
UNIVERSITY OF WISCONSIN,
MADISON 6, WISCONSIN

LITERATURE CITED

- BERGER, C. A. 1938. Multiplication and reduction of somatic chromosome groups as a regular developmental process in the mosquito *Culex pipiens*. Carnegie Inst. of Wash. No. 496:209-232.
- DARLINGTON, C. D. 1937. Recent advances in cytology. 2nd Ed. J. & A. Churchill Ltd. London.
- ERVIN, C. D. 1941. A study of polysomaty in *Cucumis melo*. Amer. Jour. Bot. 28:113-124.
- FISK, EMMA L. 1927. Chromosomes in *Zea Mays*. Amer. Jour. Bot. 14:53-75.
- GATES, R. R. 1912. Somatic mitosis in *Oenothera*. Ann. Bot. 26:993-1010.
- GENTCHEFF, G., AND GUSTAFSSON, A. 1939. The double chromosome reproduction in *Spinacia* and its cause. I. Normal behavior. II. An x-ray experiment. Hereditas 25:349-357; 371-385.
- HANCE, R. T. 1918. Somatic chromosomes of *Oenothera scintillans*. Genetics 3:225-275.
- KUWADA, Y. 1919. Die Chromosomenzahl von *Zea Mays* L. Jour. Coll. Sci. Tokyo 39:1-148.
- LANGLET, O. F. I. 1927. Zur Kenntnis der polysomatischen Zellkerne im Würzelmeristem. Svensk. Bot. Tidskr. 21:397-422.
- LONGLEY, A. E. 1927. Supernumerary chromosomes in *Zea Mays*. Jour. Agric. Res. 35:769-784.
- MONTGOMERY, T. H. 1901. A study of the chromosomes of the germ cells of Metazoa. Trans. Amer. Philos. Soc. 20:154.
- RHOADES, M. M. 1940. Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics 25:483-520.
- STOMPS, T. J. 1916. Über den Zusammenhang zwischen Statur und Chromosomenzahl bei den Oenotheren. Biol. Centralbl. 36:129-160.
- UPCOTT, M. 1939. The external mechanics of the chromosome. VII. Abnormal mitosis in the pollen grains. Chromosoma Abt. 13. Zeitsch. f. Zellf. u. mikr. Anat. 1:178-190.
- WINKLER, H. 1916. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeitschr. f. Bot. 8:417-531.

GROWTH FACTORS FOR TRICHOPHYTON MENTAGROPHYTES¹

William J. Robbins and Roberta Ma²

IN EARLIER papers from this laboratory (Butler, Robbins and Dodge, 1941; Robbins and Kavanagh, 1942; Robbins and Ma, 1941, 1942A, 1942B, 1944) specific fungi have been reported for which partial or complete deficiencies for biotin, thiamine, pyridoxine, hypoxanthine, *p*-amino benzoic acid, and *i*-inositol, singly or in combination, limited growth in a basal medium of mineral salts, sugar and asparagine. Some of these organisms appeared to suffer also from partial deficiencies for other unidentified factors. These were present in potato extract, peptone and other natural products, and were not adsorbed by Norit A. In earlier work with *Phycomyces* (Robbins and Hamner, 1940) these factors were referred to as factor Z₂; they might also be called filtrate factors. The present paper is concerned with a study of filtrate factors for *Trichophyton mentagrophytes* (*T. gypseum*) (Emmons, 1934). There is no necessary implication that these are identical with factor Z₂ for *Phycomyces*.

¹ Received for publication March 20, 1945.

² This investigation was supported in part by a grant from Dr. Charles L. Mayer whose assistance is gratefully acknowledged.

MATERIALS AND METHODS.—The strain of *T. mentagrophytes* used was isolated from a human subject in August, 1942, and identified through the courtesy of Dr. Rhoda W. Benham. Stock cultures were maintained on a thiamine-peptone agar.³ By subculturing at intervals which did not exceed two weeks the strain was kept in a freely sporulating condition. This is referred to as the normal (N) strain.

Inoculations were made by transferring a bit of mycelium (and spores if present) about the size of a pin head from stock cultures on thiamine-peptone

³ The thiamine-peptone agar consisted of the basal solution plus 1.0 g. neo-peptone, 0.5 mg. thiamine and 15.0 g. difco agar per liter. The basal solution contained per liter 1.5 g. KH₂PO₄, 0.5 g. MgSO₄·7H₂O, 2.0 g. asparagine and 50.0 g. dextrose. To this solution the following mineral supplements were added in p.p.m.: 0.005 B, 0.02 Cu, 0.10 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn. The basal agar medium was prepared by adding from 1.5 to 2.0 per cent of purified agar to the basal solution. The agar was purified by extracting difco agar with aqueous pyridine, washing with dilute hydrochloric acid and neutralizing with calcium hydroxide. The agar was then leached thoroughly with distilled water, washed with redistilled 95 per cent alcohol and dried at 50°C.

agar. In our experiments the fungus was grown in liquid media and on agar slopes in test tubes. Each tube contained 8 ml. of agar medium in a test tube 20 mm. by 120 mm. Observations on growth were made at frequent intervals and colony diameters were measured. For liquid cultures 25 ml. were used in a 125 ml. Erlenmeyer flask, and growth was measured by autoclaving the cultures, filtering into Gooch crucibles and drying at 100°C. Both agar and liquid cultures were run in triplicate. All glassware was pyrex. It was cleaned with chromic-sulfuric acid cleaning mixture and thoroughly rinsed with tap water followed by distilled water. All chemicals were of C.P. grade. The asparagine was treated with Norit A and recrystallized from alcohol.

EXPERIMENTAL RESULTS.—Relation to known vitamins.—Preliminary experiments demonstrated that the (N) strain grew slowly on the basal agar medium, but rapidly on thiamine-peptone agar. Since the beneficial effect of peptone on *T. discoides* had been found to be caused primarily by traces of thiamine, pyridoxine and *i*-inositol present in the peptone (Robbins and Ma, 1942B), it was assumed that *T. mentagrophytes* also might show deficiencies for these vitamins. However, the addition to each tube of the basal agar medium of 5 μ moles of thiamine, 5 μ moles of pyridoxine and 1 mg. of *i*-inositol singly or in combination was of no benefit. No favorable effect on growth was observed from the addition to the basal medium of a mixture of twelve vitamins or vitamin-like substances.⁴

Temperature.—*T. mentagrophytes* (N) developed over a considerable range of temperature. It grew at 11°, 15°, 20°, 25°, 30° and 35°C. Growth was most rapid at 35°C.; at 11°C. development was slow. The favorable action of peptone was observed at all temperatures. Unless otherwise noted our experiments were carried on at 35°C.

Beneficial materials other than peptone.—Hydrolysates of egg albumen, of difco gelatine, of Eastman purified gelatine, a hot-water extract of cotton batting (Robbins and Ma, 1942A), a filtrate fraction from potato tubers (Robbins and Hamner, 1940), and a vitamin-free casein hydrolysate,⁵ in addition to peptone, were beneficial though not equally so (fig. 1). When the activity of equal dry weights of these substances was compared, they fell in the following order: casein hydrolysate=>pep-

tone⁶=egg albumen hydrolysate>Eastman gelatine hydrolysate=Difco gelatine hydrolysate>cotton extract>D_R fraction.

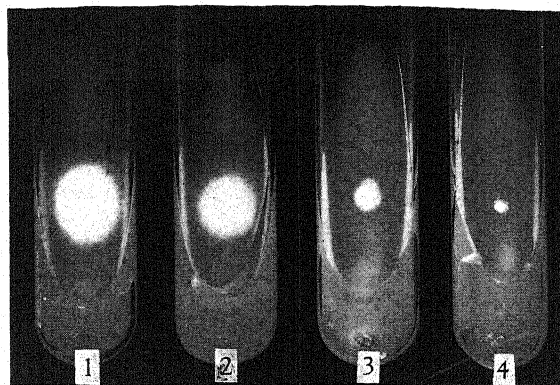


Fig. 1. *T. mentagrophytes* (N) grown 6 days on basal agar medium containing no asparagine plus (1) 10 mg. casein hydrolysate; (2) 5 mg. casein hydrolysate; (3) 10 mg. casein hydrolysate prepared from amino acids; (4) 10 mg. asparagine.

The casein hydrolysate, neopeptone or egg albumen hydrolysate was about five times as effective as gelatine hydrolysate; that is, 5.0 mg. of gelatine hydrolysate produced about the same effect on growth as 1.0 mg. of peptone, egg albumen hydrolysate or casein hydrolysate. A slight but definite beneficial effect was observed from the addition of 10.0 μ g. of casein hydrolysate per tube containing 8 ml. of the basal agar medium. The benefit increased up to 20 mg. per tube, but growth with 50 mg. was not quite as good as with 20 mg. Beneficial effects were noted with 0.1 mg. (the smallest amount used) of peptone per tube, and increased growth was obtained up to 10 mg. per tube; 100 mg. was little if any more effective than 10 mg. It is probable that the optimum amount of neopeptone per tube was 20 or 25 mg. Increased growth occurred with additions of egg albumen hydrolysate ranging from 0.1 mg. to 2.0 mg. per tube. Poorer results were obtained with 100 mg. than with 10 mg.

No benefit was observed from the addition of the vitamin mixture to the basal medium plus 10 mg. of vitamin-free casein hydrolysate per tube.

Effect of hydrogen-ion concentration.—The hydrogen-ion concentration of our basal medium was about pH 4.5. It was adjusted by phosphate buffers to 5.5, 6.0 and 6.5. One experiment was carried out using media solidified with 1.5 per cent purified agar; another in liquid cultures. For both the agar and liquid media we used the basal medium adjusted to the various hydron concentrations and the same solutions to which 1.0 mg. of casein hydrolysate or 25 mg. of peptone were added per tube or per flask. The peptone reduced the acidity of the unadjusted basal solution (from pH 4.5 to pH 5.2); the casein hydrolysate in the amount used had little effect.

⁶ The casein hydrolysate was superior to peptone when the effect of the larger amounts was compared.

⁴ These were added per tube in the following quantities: 0.05 γ biotin methyl-ester, 2.16 mg. *i*-inositol and 10 μ moles each of *p*-amino benzoic acid, calcium pantothenate, hypoxanthine, guanine hydrochloride, 2-methyl-1, 4-naphthohydroquinone diacetate, nicotinamide, pimelic acid, pyridoxine, riboflavin and thiamine.

⁵ The vitamin-free casein hydrolysate was that prepared by the S.M.A. corporation. For our use CaCO₃ was added to the preparation to neutralize the acidity, and the excess CaCO₃ was removed by filtering through ashless filter paper. The Eastman gelatine was calfskin gelatine with 0.03 per cent ash on a moisture-free basis. The gelatine and egg albumen hydrolysates were prepared by refluxing the material with 8 N H₂SO₄ for 24 hours. The H₂SO₄ was neutralized with Ba(OH)₂ and the BaSO₄ removed by filtration.

TABLE 1. Colony diameters of *T. mentagrophytes* (N) in mm. after four and ten days on agar media adjusted to the hydron concentrations given.

Basal medium			Basal medium plus 1 mg. casein hydrolysate			Basal medium plus 25 mg. peptone		
Initial pH	Colony diameter after 4 days	10 days	Initial pH	Colony diameter after 4 days	10 days	Initial pH	Colony diameter after 4 days	10 days
4.5	1.5	6.0	4.5	10.0	30.0	5.2	17.0	35.0
5.4	2.0	9.0	5.5	11.0	30.0	5.5	17.0	34.0
6.0	3.0	10.0	6.0	10.0	30.0	5.9	17.0	34.0
6.5	6.0	13.0	6.6	12.0	30.0	6.4	17.0	25.0

On the basal agar medium growth was improved by reducing the acidity from pH 4.5 to pH 6.5 (table 1). On the media supplemented with casein the differences in initial acidity had little effect on growth, and in the media supplemented with peptone growth was better in the cultures with the greater initial acidity. However, growth on the basal medium at pH 6.5 was half or less that obtained on the same medium supplemented with 1 mg. of casein hydrolysate per tube.

The cultures in the liquid media were grown for 10 days, and final hydron concentrations were determined after autoclaving. Growth in the basal medium was improved by reducing the initial acidity. The effect was less marked in the medium supplemented with casein, and not evident in those supplemented with peptone, probably because of changes in hydron concentration during growth as shown by the differences between the initial and final pH (table 2).

We concluded that the basal medium would be more satisfactory if adjusted to pH 5.5 or 6.0. However, it was evident that the beneficial effects of peptone, or of casein hydrolysate, on the growth of *T. mentagrophytes* (N) could not be accounted for by the action of these supplements on the hydron concentration of the culture media.

Relation to inorganic nitrogen.—Inorganic nitrogen in the form of NH_4NO_3 was almost completely unavailable to *T. mentagrophytes* (N). When NH_4NO_3 in amounts of 5.0 mg., 1.0 mg., 0.5 mg. or 0.05 mg. per tube was added to the basal agar medium containing no asparagine, growth was very little better than in the medium with no nitrogen. The same results were obtained when NH_4NO_3 was added to the basal liquid medium lacking nitro-

gen. Addition of 10.0 mg., 5.0 mg., 0.5 mg. or 0.05 mg. of NH_4NO_3 per flask containing 25 ml. of culture liquid gave the same dry weight as was obtained in the solution with no nitrogen.

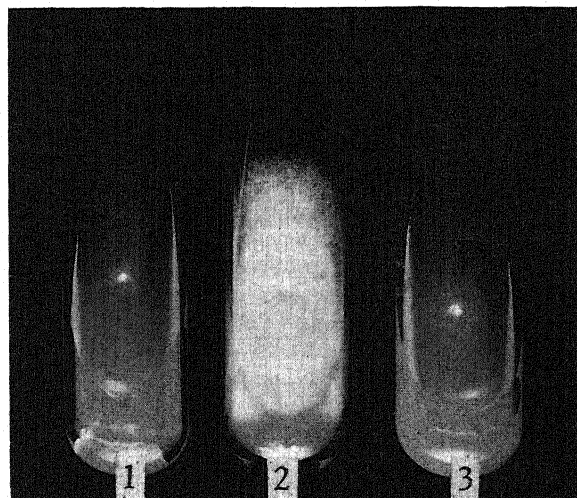


Fig. 2. *T. mentagrophytes* (N) grown 10 days on basal agar medium at pH 6.2 and containing no asparagine plus (1) no addition; (2) 10 mg. casein hydrolysate; (3) 10 mg. NH_4NO_3 .

The addition of a small amount of casein hydrolysate (1.0 mg. or less) to a solution containing NH_4NO_3 gave no better growth than the same amount of casein hydrolysate alone; in other words, the presence of casein hydrolysate did not enable the fungus to use NH_4NO_3 .

TABLE 2. Dry weight of mycelium produced by *T. mentagrophytes* (N) in ten days in liquid media adjusted to various hydron concentrations.

Basal medium			Basal medium plus 1.0 mg. casein hydrolysate			Basal medium plus 25.0 mg. peptone		
Initial pH	Final pH	Ave. dry weight mg.	Initial pH	Final pH	Ave. dry weight mg.	Initial pH	Final pH	Ave. dry weight mg.
4.5	4.5	0.1	4.6	6.0	3.3	5.2	6.4	10.4
5.5	5.4	0.2	5.5	6.2	4.0	5.5	6.4	10.3
6.0	5.8	0.4	6.0	6.2	4.0	5.9	6.4	11.4
6.5	6.0	0.8	6.5	6.2	4.2	6.4	6.4	11.3

TABLE 3. Average dry weight in mg. of triplicate liquid cultures. *T. mentagrophytes* (N) and (A) strains after fourteen days in basal solution without asparagine adjusted to pH 6.2.

Addition per flask containing 25 ml. basal solution without asparagine	Average dry weight mg.	
	(N) strain	(A) strain
No nitrogen	0.0	0.4
10 mg. casein hydrolysate.....	15.1	36.8
2 mg. casein hydrolysate.....	3.8	7.4
1 mg. casein hydrolysate.....	1.6	3.6
10 mg. NH_4NO_3	0.0	5.4
5 mg. NH_4NO_3	0.0	2.5
1 mg. NH_4NO_3	0.0	0.9
0.5 mg. NH_4NO_3	0.0	0.6

Raising the pH of the medium to 6.2 did not render NH_4NO_3 available to the (N) strain (table 3 and fig. 2).

Relative value of casein and asparagine.—The vitamin-free casein hydrolysate neutralized with calcium carbonate was much more beneficial than was asparagine (fig. 1). Asparagine or casein hydrolysate was added to the basal agar medium (containing no asparagine) adjusted with potassium hydroxide to pH 6.0. The quantities of the nitrogen source per tube were 20.0, 15.0, 10.0, 5.0, 2.5 and 1.0 mg. One mg. of casein hydrolysate was distinctly beneficial, and the benefit increased with the amount of hydrolysate added. One mg. of asparagine was much less beneficial than 1 mg. of casein hydrolysate, and increasing the amount had little or no effect on the growth.⁷ After 4 days' growth the diameter of the colonies in the tubes without nitrogen measured 2.0 mm.; in those with 1.0 mg. of casein hydrolysate, 15 mm.; and with 1.0 mg. of asparagine, 4.0 mm. At the end of one week these figures were 5.0 mm., 30.0 mm. and 9.0 mm. respectively.

Similar results were obtained in liquid culture (table 4). The addition of 20.0 mg. of casein hydrolysate per flask produced 51.0 mg. dry weight

⁷ This statement is correct for the first week or two of growth. Given sufficient time, growth with the larger amounts of asparagine (20 mg. per tube) exceeded that with smaller amounts (1 mg. per tube).

in 21 days; with 20.0 mg. of asparagine the yield was 2.2 mg. In fact, the growth as measured by dry matter was greater with 1.0 mg. of casein hydrolysate than with 20.0 mg. of asparagine (table 4).

Although growth increased in both liquid and agar media with increasing amounts of casein hydrolysate, differences in the amount of asparagine supplied had little effect. The benefit from asparagine seemed to be limited by some factor which was not effective for casein hydrolysate.

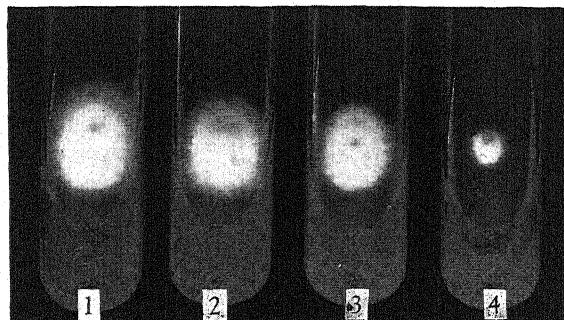


Fig. 3. *T. mentagrophytes* (N) grown 7 days on basal agar medium containing no asparagine plus (1) 10 mg. casein hydrolysate; (2) 10 mg. of equal amounts of l-leucine, d-isoleucine, dl-phenylalanine, d-glutamic acid, l-tyrosine and d-arginine; (3) 10 mg. l-leucine, d-isoleucine, l-tyrosine and d-glutamic acid in the proportions found in casein; (4) 10 mg. synthetic casein hydrolysate.

TABLE 4. Average dry weights of triplicate cultures of *T. mentagrophytes* (N) in 25 ml. of a liquid medium of mineral salts and sugar to which the quantities given of casein hydrolysate, of asparagine, or of a synthetic casein hydrolysate composed of amino acids were added.

Addition in mg.	Ave. dry wt. mg. after 10 days		Ave. dry wt. mg. after 21 days		
	Casein hydrolysate	Asparagine	Casein hydrolysate	Asparagine	Synthetic casein hydrolysate
20.0	28.6	0.5	51.0	2.2	21.1
15.0	21.6	0.5	32.5	2.0	15.2
10.0	11.2	0.4	14.4	2.0	12.2
5.0	6.3	0.4	8.4	2.0	7.2
2.5	4.8	0.3	6.0	1.9	5.4
1.0	2.6	0.2	3.0	1.0	2.1
0.0	0.2	0.2	0.2

Substitution of amino acids for casein hydrolysate.—Since hydrolysates of egg albumen, of highly purified gelatine and of casein, were all beneficial, it seemed probable that the active substances were amino acids. An approximation of casein hydrolysate was prepared from amino acids.⁸ This was treated with an excess of CaCO_3 and filtered. Added to the basal liquid medium without asparagine in amounts ranging from 1.0 to 20.0 mg. per flask, the synthetic casein hydrolysate was less effective than the natural casein hydrolysate, but considerably more beneficial than equal quantities of asparagine (fig. 1 and table 4). Similar results were obtained when synthetic casein hydrolysate was added to the basal agar medium without asparagine (fig. 4). Not only was less growth obtained with the synthetic hydrolysate than with the natural hydrolysate, but the character of the growth differed. With the synthetic hydrolysate the edges of the colony were sharply defined and the spread of hyphae in the agar was definitely limited. The synthetic casein hydrolysate appeared to contain injurious as well as beneficial factors.

Attempts to concentrate the beneficial material in casein hydrolysate.—The difference in the effects

⁸ The synthetic casein hydrolysate was prepared by adding to 100 ml. of distilled water the following amino acids:

Glycine, Pfanstiehl	5.0 mg.
dl-alpha-alanine Merck	19.0
dl-valine Pfan.	75.0
l-leucine Pfan.	48.5
d-isoleucine Eastman	48.5
dl-phenylalanine Univ. of Illinois	39.0
l-tyrosine Pfan.	66.0
l-tryptophane Pfan.	22.0
dl-threonine Merck	35.0
d-glutamic acid Pfan.	218.0
l-aspartic acid East.	41.0
l-proline East.	90.0
l-hydroxyproline Merck	2.0
dl-serine East.	50.0
l-cystine Pfan.	3.0
l-methionine East.	34.0
d-arginine HCl Pfan.	38.0
d-histidine HCl Pfan.	25.0
d-lysine (HCl) ₂ Pfan.	60.0

of natural and synthetic casein hydrolysate might result from the presence in the casein of some beneficial material not included in the synthetic hydrolysate, or to the presence of something detrimental in the synthetic preparation not present in the hydrolysate of the natural casein.

Attempts to concentrate beneficial material from the casein hydrolysate by solubility in ethyl alcohol were not successful. The desiccated hydrolysate was extracted with 95 per cent alcohol, the residue was extracted with 80 per cent alcohol, and the material insoluble in 80 per cent alcohol was dissolved in water. All three fractions were active but none was as active as the original casein hydrolysate when the effects of equivalent amounts were compared. The material soluble in 95 per cent alcohol had 0.1 or 0.2 the activity of the original hydrolysate, and that insoluble in 95 per cent alcohol, but soluble in 80 per cent alcohol was about one-twentieth as active as the casein hydrolysate. The material insoluble in 80 per cent alcohol had the least activity.

Extracts of casein hydrolysate were made with butyl alcohol (normal butanol) and with octyl alcohol (2-ethyl-hexyl-alcohol). The butyl alcohol extract was beneficial but less so than equivalent amounts of casein hydrolysate. The octyl alcohol extracts also were active. Added to the basal agar medium containing asparagine or the synthetic casein hydrolysate, the extracts made with octyl alcohol increased the spread of growth (fig. 4); benefit was observed with as little as 5 μg . dry matter. Octyl alcohol evaporated to dryness, and taken up in water, had no effect. However, casein hydrolysate extracted with octyl alcohol lost little or none of its activity, and octyl alcohol extracts had little or no effect on the dry weight in liquid cultures of the basal solution containing synthetic casein hydrolysate. It appeared, therefore, that whatever was extracted by the octyl alcohol was not responsible for the differences in dry weight noted between

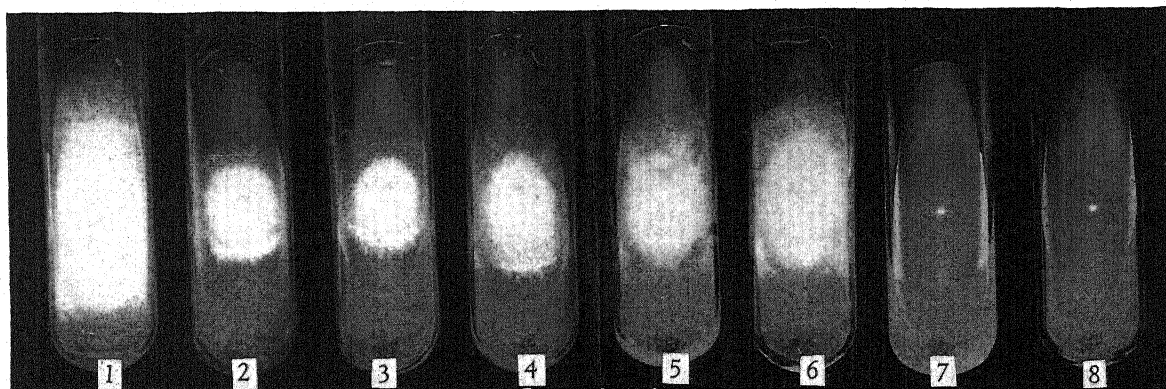


Fig. 4. *T. mentagrophytes* (N) grown 11 days on basal agar medium containing no asparagine plus (1) 10 mg. casein hydrolysate; (2) 10 mg. synthetic casein hydrolysate; (3, 4, 5 and 6) 10 mg. synthetic casein hydrolysate plus octyl alcohol extract of natural casein hydrolysate (0.01 mg., 0.1 mg., 1 mg. and 10 mg. casein hydrolysate); (7) octyl extract of 10 mg. casein hydrolysate; (8) no addition.

media containing the natural and the synthetic hydrolysates.

Activity of individual amino acids.—Failure to obtain a concentrate which would account for the beneficial effect of casein hydrolysate and the evidence for injury shown by a casein hydrolysate prepared from amino acids suggested that the effect of the various amino acids should be determined individually. Each of nineteen amino acids was added in 1.0 mg. quantities to the basal agar medium containing no asparagine. The experiment was performed in triplicate. For comparison, tubes containing 1.0 mg. of neopeptone, 1.0 mg. of asparagine and 1.0, 0.5, 0.25 or 0.1 mg. of casein hydrolysate were included. Each amino acid and the casein hydrolysate was treated with an excess of calcium carbonate, and filtered before use. The asparagine and the neopeptone were not. General observations on growth, measurements of colony diameters, and comparisons with the development in tubes containing casein hydrolysate were made at intervals of four or five days. It was found that *T. mentagrophytes* grew with any one of at least fourteen amino acids as a source of nitrogen. No one of these, however, was as good when 1.0 mg. was supplied per tube as was an equal weight of casein hydrolysate.

The effectiveness of the amino acids varied. Some were more beneficial for surface growth, others for subsurface growth. Some induced the formation of pigment, others did not. With some the colonies developed finger-like radiating processes, with

others the colony had a uniform edge. The relative value of the amino acids changed somewhat with the age of the cultures. The differences in the character of the growth (thickness as compared to extent, surface growth as compared to subsurface growth) made a comparison of the activity of the various acids difficult, and colony diameters alone (table 5) were not entirely satisfactory for such evaluations.

After six days incubation, observations and comparisons showed that growth with leucine, isoleucine, glutamic acid, and tyrosine was best followed by phenylalanine. Aspartic acid, proline, arginine, and histidine were beneficial, but less so than phenylalanine. Growth with glycine, valine, and α -alanine was slightly superior to that on the medium without nitrogen where a small amount of development occurred at the expense of nitrogen carried over with the inoculum. However, even the best growth with 1.0 mg. of a single amino acid was less than that with 0.25 mg. of casein hydrolysate.

After eleven days six amino acids were distinctly beneficial in the following order: tyrosine>glutamic acid>isoleucine>leucine=phenylalanine>arginine. The effects with proline and aspartic acid were as great as those obtained with asparagine. Growth with glycine, α -alanine, valine and histidine was superior to that on the medium with no nitrogen but less than that obtained with asparagine. Cystine induced a spreading subsurface growth so sparse as to be unnoticed unless the tubes were carefully

TABLE 5. Colony diameters in mm. of *T. mentagrophytes* (N) on a basal agar medium containing no asparagine to which 1 mg. of the amino acids given were added per tube.

Additions to basal agar medium containing no asparagine	Colony diameter in mm.			
	4 days	7 days	14 days	20 days
glycine	2.5	6.0	20.0	27.0
dl- α -alanine	2.5	3.5	14.0	22.7
dl-valine	2.3	4.0	12.3	21.3
l-leucine	5.2	10.0	21.0	34.0
d-isoleucine	4.7	9.2	26.0	40.0
dl-phenylalanine	3.2	8.7	29.0	40.3
l-tryptophane	2.0	2.2	8.3	16.3
dl-threonine	2.0	2.0	3.0	6.0
d-glutamic acid	4.2	9.5	22.0	43.3
dl-aspartic acid	2.5	4.0	15.0	33.7
l-hydroxyproline	2.0	2.0	6.0	14.7
dl-serine	2.0	2.7	9.3	21.0
l-cystine HCl	1.5	2.2	19.7	28.0
l-methionine	1.5	2.0	9.3	23.7
l-tyrosine	3.8	9.5	27.3	40.0
l-lysine (HCl) ₂	1.5	2.0	5.2	10.7
l-proline	2.5	6.0	22.3	39.3
d-arginine HCl	2.5	6.2	22.0	36.7
l-histidine HCl	2.5	4.5	15.7	28.7
neopeptone	9.7	20.3	40.0	46.0
asparagine	2.0	4.2	14.7	27.3
1 mg. casein hydrolysate	10.2	19.7	38.0	48.3
0.5 mg. casein hydrolysate	8.0	15.0	31.3	39.3
0.25 mg. casein hydrolysate	8.2	15.0	30.7	40.0
0.1 mg. casein hydrolysate	3.5	9.0	24.0	34.7
no addition	1.5	2.7	10.0	17.0

examined; the surface growth was slight. Serine had no effect. Growth with five amino acids (tryptophane, threonine, hydroxyproline, methionine and lysine) was less than that on the medium without nitrogen; i.e., these five amino acids as judged by growth in diameter were injurious. The best growth with 1.0 mg. of an amino acid about equaled that with 0.25 mg. of casein hydrolysate.

By the end of 34 days arginine was most effective; growth with 1.0 mg. of this amino acid nearly equaled that with 1.0 mg. casein hydrolysate. Glycine, alanine, valine, leucine, glutamic acid, serine, tyrosine, lysine and proline were approximately equal to 0.5 mg. of casein. Isoleucine, phenylalanine, aspartic acid and histidine were superior to 0.25 mg. casein but poorer than 0.5 mg. Growth with methionine was equivalent to that with 0.1 mg. casein. Tryptophane, threonine, hydroxyproline and cystine were no better or poorer than the medium with no nitrogen where a thin spreading growth developed largely in the agar.

The improvement in growth with some of the amino acids relative to casein hydrolysate observed after 34 days incubation was largely because growth continued in the media containing the amino acids but had reached its maximum some time before in the tubes containing casein hydrolysate. In other words, the nitrogen content became a limiting factor earlier in the media containing casein hydrolysate, where growth was more rapid, than it did in those media containing amino acids, where growth was slower.

The difficulty in evaluating the effects of the various amino acids is emphasized by the differences in the order in which they were placed from the comparisons summarized above, and from the colony diameters in table 5. For example, our observations after eleven days led us to list tyrosine, glutamic acid, isoleucine, leucine, phenylalanine and arginine as the six best amino acids. From colony measurements at the end of fourteen days proline, glycine and cystine would be included in this group, although the character of growth was such that we excluded them from the six best, placing proline and glycine in the same class as asparagine, and cystine next to those which had no effect or were injurious. From colony diameters at the end of fourteen days the effects of histidine and alanine equaled or exceeded that of asparagine, but from our observations on colony appearance we rated them below asparagine.

In considering the effect of the individual amino acids it should be pointed out that 1.0 mg. of a single amino acid, except for glutamic acid, equals or exceeds the amount of that acid in 10.0 mg. of casein hydrolysate. If the beneficial action of casein hydrolysate was caused by a single amino acid then 1.0 mg. of an amino acid, except glutamic acid, should have been more effective than 10.0 mg. of casein hydrolysate; 1.0 mg. of glutamic acid should have had the effect of about 5.0 mg. of casein hydrolysate. However, in no instance was a single

amino acid as effective as an equal weight of casein hydrolysate.

T. mentagrophytes (N) developed a yellow pigment in the medium containing alanine, a reddish brown color with glycine, valine, histidine and serine, and a brownish color with arginine and asparagine.

Colonies with finger-like process in the agar developed with alanine, glycine, leucine, valine and serine. The colonies on media containing aspartic acid, isoleucine, glutamic acid, lysine, methionine, tyrosine, phenylalanine, casein hydrolysate, and peptone, were more or less similar in appearance with smooth and regular edges. Those with arginine, asparagine, and histidine, were intermediate.

Amounts larger or smaller than 1.0 mg. of some of the amino acids were used in other experiments. For example, leucine at 0.485 mg. per tube (an amount equal to that in several mg. of casein hydrolysate) had an effect equal to between 0.25 and 0.5 mg. of casein hydrolysate. Glutamic acid at 2.18 mg. per tube (the amount in 10 mg. of casein hydrolysate) was about as effective as 1.0 mg. of casein hydrolysate.

Effect of mixtures of amino acids.—Since none of the amino acids used singly had as great an effect on *T. mentagrophytes* (N) as an equal amount of casein hydrolysate, it seemed desirable to test mixtures of selected amino acids. To group the amino acids according to their effectiveness was difficult because, as was pointed out earlier, the relative value of the various amino acids changed somewhat with time and the differences in the character of growth on an agar medium (thickness compared to spread, surface growth compared to subsurface growth) obtained with the different amino acids made evaluation difficult. However, on one criterion or another various mixtures of amino acids were prepared and tested. For example, the appearance of the colonies at the end of five weeks, modified to some extent by observations made during the earlier development, was used to divide the 19 amino acids into four groups. These were: (1) the seven best acids including arginine, glycine, α -alanine, leucine, tyrosine, lysine⁹ and proline; (2) the five next best (valine, isoleucine, glutamic acid, aspartic acid and histidine); (3) the two next best (serine and phenylalanine); (4) the five least effective (tryptophane, threonine, hydroxyproline, cystine and methionine).

Each of these four groups was added to the basal agar medium without asparagine in 1.0 mg. and 10.0 mg. quantities per tube; the total amount was equally divided amongst the acids in a group. For example, 10.0 mg. of group 1 was composed of 1.4 mg. of each of the seven amino acids, but 10.0 mg. of group 3 was composed of 5.0 mg. of each of the two amino acids. In addition, groups 1 and 2 were combined, making twelve amino acids; groups 1, 2

⁹ Although lysine was not favorable for growth in diameter, it was included in this group because of its effect on surface growth.

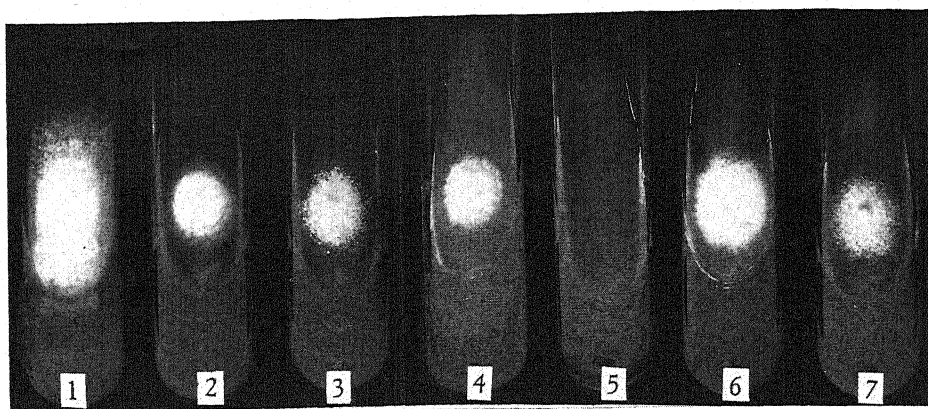


Fig. 5. *T. mentagrophytes* (N) grown 10 days on basal agar medium containing no asparagine plus (1) 10 mg. casein hydrolysate; (2) 10 mg. of 7 amino acids in group 1; (3) 5 amino acids in group 2; (4) 2 amino acids in group 3; (5) 5 amino acids in group 4; (6) 14 amino acids in groups 1, 2 and 3; (7) 19 amino acids in groups 1, 2, 3 and 4. See text for details.

and 3 were combined (14 amino acids); and groups 1, 2, 3 and 4 were combined (19 amino acids). The larger the number of amino acids added per tube the smaller was the quantity of each amino acid present. Observations on growth were made over a period of 5 weeks (table 6).

The five amino acids in group 4 were distinctly detrimental (fig. 5). At 10.0 mg. per tube they inhibited growth completely for the first week or ten days, and development after two weeks was slight. At 1.0 mg. per tube also this group was inhibitory; very little growth occurred in the first week and growth was slow thereafter. The addition of these five amino acids to the fourteen others reduced growth about one-half for the 10.0 mg. quantity

(fig. 5), and was definitely harmful when 1.0 mg. was used.

All of the other mixtures were beneficial. The character of growth (thickness, spread, surface, subsurface) varied with the various combinations used, and no group equaled in effectiveness an equal weight of casein hydrolysate. Growth with 10.0 mg. of each group was better than with 1.0 mg., with the exception of group 4. The combination which approached casein hydrolysate most closely in character and amount of growth was that containing the 14 amino acids. The effect of 10.0 mg. of the 14 amino acids was equivalent to that of 2.0 mg. of casein hydrolysate.

Other combinations of amino acids were tested. Nine amino acids (leucine, isoleucine, tryptophan, glu-

TABLE 6. Colony diameter of *T. mentagrophytes* (N) on basal agar medium containing no nitrogen and supplemented with casein hydrolysates or 19 amino acids in groups. See text for details.

Addition to basal medium containing no asparagine	Average colony diameter in mm.		
	4 days	7 days	14 days
Casein hydrolysate 10 mg.....	18.3	35.0	58.0
Casein hydrolysate 5 mg.....	16.3	32.7	52.3
Casein hydrolysate 2 mg.....	12.7	27.0	48.7
Casein hydrolysate 1 mg.....	12.7	25.3	40.7
Casein hydrolysate 0.5 mg.....	13.0	24.7	40.0
No addition	2.0	5.0	29.6
Group 1 7 amino acids 10 mg.....	8.2	14.7	32.7
Group 1 7 amino acids 1 mg.....	6.5	10.3	27.0
Group 2 5 amino acids 10 mg.....	10.0	14.7	36.0
Group 2 5 amino acids 1 mg.....	7.0	12.3	27.0
Group 3 2 amino acids 10 mg.....	11.3	19.0	34.7
Group 3 2 amino acids 1 mg.....	10.0	15.7	31.3
Group 4 5 amino acids 10 mg.....	0.0	0.0	2.3
Group 4 5 amino acids 1 mg.....	0.0	2.0	9.3
Group 1 and 2 10 mg.....	10.3	17.0	44.0
Group 1 and 2 1 mg.....	11.0	15.0	30.3
Group 1, 2 and 3 10 mg.....	12.7	25.0	43.3
Group 1, 2 and 3 1 mg.....	10.3	17.3	26.3
Group 1, 2, 3 and 4 10 mg.....	10.0	17.0	33.3
Group 1, 2, 3 and 4 1 mg.....	6.0	14.7	26.0

tamic acid, phenylalanine, aspartic acid, proline, arginine and histidine) were used in the proportions found in casein in amounts per tube equivalent to 10.0, 1.0, 0.5, 0.25 and 0.1 mg. of casein hydrolysate. They were added to the basal agar medium without asparagine. For the first week of growth, 6.14 mg. (equivalent to 10 mg. of casein hydrolysate) of the nine amino acids nearly equaled the activity of 5.0 mg. of casein hydrolysate. However, the smaller quantities were relatively less effective. For example, 0.614 mg. (equivalent to 1.0 mg. of casein hydrolysate) approximately equaled the effect of 0.25 mg. of casein hydrolysate, and 0.307 mg. (equivalent to 0.5 mg. of casein hydrolysate) had about the same effect as 0.1 mg. of the hydrolysate. These nine amino acids furnished in the proportions found in casein hydrolysate were, therefore, between one-third and three-fourths as active as equal amounts of casein hydrolysate, and between one-fifth and one-half as beneficial as equivalent amounts of casein hydrolysate.

Four amino acids (leucine, isoleucine, tyrosine and glutamic acid) were used in the proportions found in casein hydrolysate in amounts equivalent to 10.0, 1.0 and 0.25 mg. of casein hydrolysate per tube of the basal agar medium without asparagine. When equal weights were compared, these four amino acids were about as effective as the nine described above. When amounts equivalent to casein hydrolysate were compared the four amino acids were somewhat less effective; the extent of growth was as great or greater than was obtained with the nine amino acids, but the growth was less heavy.

A mixture of six amino acids (leucine, isoleucine, phenylalanine, glutamic acid, tyrosine and arginine) was used in 10.0 mg. amounts per tube of the

more beneficial than 10.0 mg. of the nine amino acids, or of the four amino acids previously used.

The six amino acids mentioned above, used in equal amounts, seemed to be the best mixture tested (fig. 3). The effect of omitting one after the other of the six acids leaving the other five was determined. The omission of leucine had little effect. The omission of arginine and of tyrosine reduced growth most, followed by glutamic acid and then by phenylalanine and isoleucine.

We also added to the six amino acids α -alanine, valine, serine, histidine and lysine singly in the amounts found in 10 mg. of casein. Valine (0.75 mg.) alone seemed to be of any benefit; serine had no effect; α -alanine (0.2 mg.), histidine (0.25 mg.) and lysine (0.6 mg.) reduced growth somewhat. When 10.0 mg. of the eleven amino acids¹⁰ were used in the proportions found in casein, growth was poor, about one-half of that obtained with 10 mg. of the six amino acids.

Taurocholate and glycocholate.—Sodium taurocholate and sodium glycocholate proved to be poor sources of nitrogen. They were added in quantities of 1.0, 2.5, 5.0, 7.5 and 10.0 mg. per tube of the basal agar medium lacking asparagine. Growth with taurocholate was somewhat better than with glycocholate, but the best growth with either did not approach that with 1.0 mg. of casein hydrolysate.

Pleomorphic strains.—As cultures of *T. mentagrophytes* age, pleomorphic forms develop.¹¹ These "mutants" are characterized by white aerial cottony mycelium and sparse conidial production. Such pleomorphic forms are said to be less infectious than the normal form and to be stable; that is, they do not revert to normal (Brumpt, 1927).

Pleomorphisms appeared in our experiments on various parts of the colonies of the (N) form and rapidly overgrew them (fig. 6). We isolated three pleomorphic forms from the (N) strain and used them in part of our studies. One of these (A) grew vigorously on the basal agar medium, formed little pigment and was nearly sterile; the second (B) grew less vigorously though more so than (N), formed a few conidia and produced a reddish brown pigment in both liquid and agar culture; the third (C) was weakly pleomorphic and differed little from (N). We maintained strains (A) and (B) apparently unchanged for more than 14 months by subculturing at intervals of two weeks or less. At some time during this period and after the experiments described here strain (C) underwent further modification in the direction of (A). From our experience it would appear possible to obtain a large

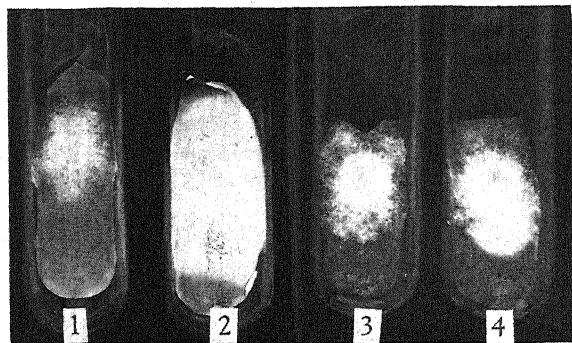


Fig. 6. *T. mentagrophytes*. 1 and 2 on basal agar medium; 3 and 4 on basal agar medium plus 0.1 mg. casein hydrolysate. In (1) the culture is normal; in (2) fully pleomorphic; in (3) normal and in (4) pleomorphism has begun on lower right edge of colony. Age 27 days.

basal agar medium without asparagine. In one experiment the six acids were used in the proportions found in casein and in another in equal quantities, that is, 1.66 mg. of each. The six amino acids in equal amounts were somewhat more beneficial than in the proportions found in casein, and they were

¹⁰ These were leucine, isoleucine, phenylalanine, glutamic acid, tyrosine, arginine, α -alanine, valine, serine, histidine and lysine.

¹¹ Medical mycologists have given the term pleomorphism a special meaning. We have used it here in the specialized sense. It is uncertain whether the so-called mutations or saltations observed for other fungi are comparable to the pleomorphisms of the dermatophytes (Emmons, 1932).

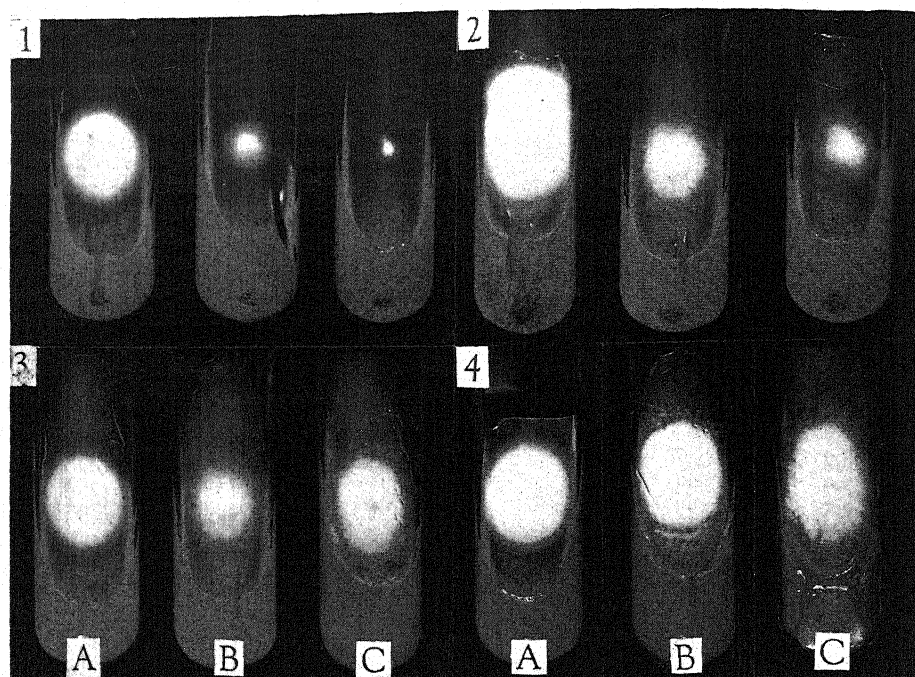


Fig. 7. Three pleomorphic forms of *T. mentagrophytes* (A), (B) and (C). (1) The three forms grown on basal agar medium; (2) on basal agar medium plus 1.0 mg. of casein hydrolysate; (3) (A) on basal medium, (B) on basal medium plus 1.0 mg. casein hydrolysate, (C) on basal medium plus 10 mg. casein hydrolysate; (4) (A) on basal medium plus 0.01 mg. casein hydrolysate, (B) on basal medium plus 10.0 mg. casein hydrolysate, (C) on basal medium plus 25 mg. peptone. Age 7 days.

number of pleomorphic strains varying in vigor and forming a graded series (fig. 7).

Effect of casein hydrolysate on pleomorphic forms.—Casein hydrolysate and peptone benefited the growth of the pleomorphic strains (fig. 7). By the addition of suitable amounts of casein hydrolysate or peptone to the basal agar medium the spread of growth of (B) and (C) could be increased to that of (A) without such supplements. (A) grew as well without casein as (C) did with the addition of 10 mg. of casein hydrolysate, and somewhat better than (B) with the addition of 1.0 mg. of hydrolysate per tube (fig. 7). However, even with supplements of casein hydrolysate, the various strains could readily be distinguished from one another by the general morphology of the colonies.

Similar results were obtained in liquid cultures. Strains (A), (B) and (N) were grown for ten days

in triplicate in 25 ml. quantities of the basal solution to which 0.1, 1.0 or 10.0 mg. of casein hydrolysate or 25.0 mg. of peptone were added per flask. Dry weights were determined after ten days' growth (table 7). The (N) strain produced 0.1 mg. of dry matter in the basal solution, the (B) strain 3.3 mg., and the (A), 11.4 mg. The dry weight produced by the (N) strain with 25.0 mg. of peptone was about the same as that formed by the (A) strain in the basal medium. Casein hydrolysate and peptone increased the growth of all three strains.

Temperature and development of pleomorphisms.—Pleomorphisms developed more quickly at the higher temperatures and were delayed at the lower temperatures. Of fifty cultures on thiamine-peptone agar all had become pleomorphic after five weeks incubation at 35°C. Of fifty cultures at 15° no

TABLE 7. Average dry weight of triplicate cultures of *T. mentagrophytes* strains (A), (B) and (N) after ten days growth in 25 ml. of basal solution supplemented as indicated.

Additions to basal solution	Average dry weight mg.		
	(A) strain	(B) strain	(N) strain
10.0 mg. casein hydrolysate.....	49.6	32.0	17.3
1.0 mg casein hydrolysate.....	27.7	5.7	1.8
0.1 mg. casein hydrolysate.....	24.7	6.3	0.4
25.0 mg. peptone.....	47.1	23.5	10.8
None	11.4	3.3	0.1

pleomorphisms were demonstrable after nine weeks, and not over ten after thirteen weeks incubation.

Use of inorganic nitrogen by pleomorphic form.—Not only was pleomorphic form (A) found to grow more rapidly with asparagine or casein hydrolysate as a source of nitrogen, but it was able to utilize inorganic nitrogen in the form of NH_4NO_3 though much less effectively than casein hydrolysate. Ten mg. of NH_4NO_3 were about as satisfactory as 1 mg. of casein hydrolysate when observations were made in liquid or agar cultures after a period of two weeks (table 8).

Growth-promoting material produced by pleomorphic strains.—The more vigorous development of the pleomorphic strains suggested that they produced some growth-promoting material in greater amounts than the (N) strain. It was found that water-soluble thermostable substances with growth-promoting properties developed in cultures of *T. mentagrophytes*, and the effectiveness of extracts of the more vigorous pleomorphic strains was greater than that of the less vigorous strains.

For example, strains (A), (B) and (N) were grown in 25 ml. quantities of the basal liquid medium at 30°C. At the end of twelve days sufficient purified agar to make two per cent was added to each flask and the cultures were autoclaved. The mycelium was not removed. Triplicate flasks in which strain (A) had grown were inoculated with (A), another set with (B), and a third with (N). The same procedure was followed with the flasks in which (B) had grown, and in which (N) had grown.

At the end of one week the (A) strain had formed the largest colonies and the (N) the smallest (table 8). The growth of the (N) strain was increased by extracts from (A), (B) and (N), most by (A) and least by (N). The (B) strain was favorably affected by the extracts of the (A) and (B) strains, more by the former than the latter, but was not improved by extracts of (N). The (A) strain was favorably influenced by the extract of (A); it was not affected by extracts of (B) or (N). At the end of eleven days the growth of the (N) strain on (A) was scanty, but covered about one-third of the agar surface, on (B) it was about one-half as much, on (N) it was a little better than on the check, and about one-fourth of the growth on (B). Growth of the (B) strain on the medium supplemented with extracts of (A) was heavy, covering about one-third of the surface; growth on (B) was much like that

on (A) but less in extent; growth on (N) and on the check were much alike and about one-half that on (B). The growth of (A) was best on (A) forming a heavy white mycelium which covered the entire surface of the agar; on (B), (N) and the check, growth was about alike and perhaps one-half of that on (A). In general each strain was benefited by extracts of its own growth, and of those strains which were more strongly pleomorphic, but not by those less strongly pleomorphic. These results suggest that the extracts from the various strains differ qualitatively. Strain (B) appeared to produce the beneficial materials formed by (N) and some additional ones with the result that (B) was not benefited by extracts of (N). Similarly (A) appeared to produce the beneficial materials made by (N) and by (B) plus some not made by (N) or (B).

It was found also that extracts of the mycelium of (A) had more effect on the growth of (N) than extracts from an equal weight of the mycelium of (B). Five cultures of each strain, (A), (B) and (N), grown 20 days in the basal liquid medium were autoclaved, filtered through Gooch crucibles, and the mycelium dried and weighed. The weight of the mycelium from the five cultures of (A) was 785.4 mg., of (B) 570.0 mg., and of (N) 1.2 mg. The filtrate from each strain was saved, evaporated to 50 ml. and the hydrogen-ion concentration adjusted to pH 5.3. Some of the original culture fluid was treated in the same fashion. Aliquots of these solutions were added to the basal solution adjusted to pH 5.3, solidified with two per cent purified agar and tubed. The effect of the extract from 33.3 mg. of the mycelium of (A) was compared with that from 33.3 mg. of (B). Half these quantities also were used. The tubes were inoculated with the (N) strain and growth noted over a period of about three weeks. The extract from a given weight of mycelium of (A) was more beneficial than that from an equal weight of (B) (fig. 8). The differences were more pronounced in the early stages of development than later.

Similar experiments were performed using liquid cultures throughout. In one instance cultures of (A), (B) and (N) ten days old were autoclaved and filtered as before. The mycelium in five cultures of (A) weighed 55.6 mg., of (B), 32.3 mg. and of (N), 2.7 mg. The extracts were adjusted to pH 5.2. Extract from 10 mg. of the mycelium of (A) was added to the basal liquid medium and inocu-

TABLE 8. Colony diameters after seven days of *T. mentagrophytes* strains (N), (B) and (A) on basal medium in which strain (A), (B) or (N) had been grown for 12 days.

Basal medium in which there had grown strain	Average colony diameters in mm. of		
	(N) strain	(B) strain	(A) strain
(A)	15.0	21.0	31.0
(B)	15.0	17.5	21.0
(N)	5.7	9.5	21.0
None	4.0	10.0	22.0

lated with (N). The same procedure was followed for the extract of the mycelium of (B). At the end of ten days the mycelium in two cultures of the (N) strain in the (A) medium weighed 3.0 mg., in the (B) medium 2.8 mg. and in the check 0.4 mg.

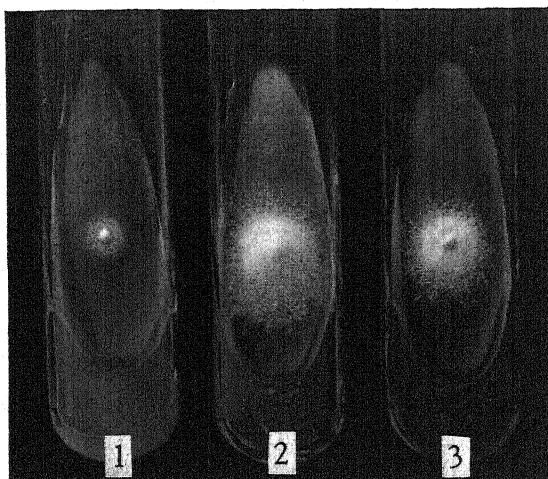


Fig. 8. *T. mentagrophytes* (N) grown 9 days on basal agar medium (1) with no addition; (2) plus extract of 20 day old culture of strain (A) equivalent to 33 mg. mycelium; (3) plus extract of 20 day old culture of strain (B) equivalent to 33 mg. mycelium.

Reversion from pleomorphic condition to normal.

—A reversion of the pleomorphic forms to the normal form was not observed. Media to which high concentrations of an extract of difco agar were added had no effect. Sulfanilamide in amounts ranging from 0.01 mg. to 5.0 mg. per tube and a medium saturated with sulfapyridine did not induce the pleomorphic form to return to a normal condition. Negative results were obtained with the basal medium to which calcium or potassium propionate was added in amounts ranging from 0.1 mg. to 50 mg. per tube. Strains (A) and (B) were incubated at 15°C. Subcultures made from each after one month were pleomorphic. No effect on reversion was observed when the pleomorphic form (A) was grown on the basal medium supplemented with 1 mg. or 5 mg. per tube of tryptophane, threonine, hydroxyproline, serine, methionine, tyrosine, phenylalanine or isoleucine. Steinberg and Thom (1942), and Steinberg (1944), obtained reversions of a nitrate-induced injury-mutant of *Aspergillus niger* when grown on certain amino acids or mixtures of amino acids.

DISCUSSION.—The questions considered in this investigation fall into two groups: those concerned with the nutrition of the normal form of *T. mentagrophytes*, and those concerned with the mutant or pleomorphic forms which originate spontaneously from the normal form.

We were originally interested in determining why the addition of peptone to a basal medium of minerals, asparagine and dextrose so markedly im-

proved the growth of *T. mentagrophytes*. The investigation of this question raised others, some of which are discussed below.

It is probable that the beneficial effects of peptone were caused by the amino acids it supplied. This conclusion appears to follow from the following considerations. The beneficial material in peptone was not adsorbed by Norit A; that is, it consisted of filtrate factors. This eliminated many of the water soluble B vitamins and, furthermore, the addition of a considerable number of vitamins to the basal medium did not affect the growth of the fungus. It appeared to be autotrophic as far as vitamins are concerned.¹² On the other hand, hydrolysates of egg albumen, of vitamin-free casein, and of a highly purified gelatine, had effects very similar to those of peptone. In fact, we prepared mixtures of amino acids which stimulated growth as peptone did though not to the same extent. Although it seems quite clear that at least part of the favorable action of peptone can be ascribed to amino acids we can not say that they are the only substances concerned. An unqualified statement to this effect would not be justified until a mixture of amino acids is devised which is as active as an equivalent amount of peptone or of casein hydrolysate; we were not successful in preparing such a mixture.

One of the interesting observations made in the course of our experiments was that *T. mentagrophytes* (N) could use asparagine, or any one of fourteen amino acids, as a source of nitrogen, but was unable to utilize ammonia. It appears that this fungus transforms asparagine, or any one of several amino acids, into all the various amino acids required for the construction of its protoplasmic proteins without previous ammonification. Of course, it is possible to conceive of some common denominator other than ammonia (hydroxylamine, for example), into which these various organic nitrogenous compounds were transformed before they were used. We are inclined to believe, however, that the normal form of *T. mentagrophytes* lacks the metabolic machinery necessary for making organic nitrogen from ammonia, at least in quantity adequate for significant growth. The situation seems to agree with the more recent concepts on the metabolism of amino acids by animals.¹³

¹² Schopfer and Blumer (1942) concluded that the benefit of certain peptones for *Trichophyton album* could be ascribed to the biotin content of the peptones. This is similar to the situation Robbins and Ma (1942b) found for *T. discoides*, except that thiamine, pyridoxine and *D*-inositol were the vitamins concerned. However, *T. discoides* suffered also from partial deficiencies for unidentified factors present in peptone, casein hydrolysate, hydrolyzed egg albumen, gelatine and other natural products. Robbins and Ma considered these unidentified favorable factors were probably amino acids, but did not succeed in demonstrating this assumption.

¹³ In animal physiology the concept of amino acids as static building stones from which proteins are made has been replaced by one which regards them as labile structures which are continuously "disappearing, reappearing and changing into each other" (Almquist, 1944). Methionine may be transformed into cystine; the carbon chain

Although in some respects *T. mentagrophytes* resembles an animal in its relation to sources of nitrogen, there appear to be no "indispensable" amino acids in the sense that the fungus failed to grow unless a particular amino acid was furnished in the nutrient medium. The power of *T. mentagrophytes* to transform organic nitrogen into protoplasmic amino acids seems to be greater than that of the higher animal.

Although *T. mentagrophytes* is capable of making from a single amino acid all those needed for its proteins, a mixture of amino acids was superior to an equal amount of any single one. This is probably because amino acids, if available in the medium, are incorporated into fungus protein more rapidly than they are supplied by the metabolic transformation of asparagine, or of a single amino acid. In other words, the mechanism for transforming asparagine or a single amino acid into those required for cell substance works too slowly. The problem with which we are concerned is somewhat different from the utilization of amino acids by *Aspergillus niger* studied by Steinberg (1942). This fungus is capable of assimilating ammonia, and it is uncertain to what extent the amino acids are used directly, and how far their utilization depends upon the ability of the fungus to form ammonia from them.

An interesting observation, which deserves further investigation, was the toxicity of some of the amino acids (threonine, methionine, hydroxyproline, and perhaps others). This was evident when these acids were used alone and, also, when they were mixed with others which were beneficial by themselves. It was especially marked for hydroxyproline which under some conditions markedly inhibited growth at 1 to 8000, and almost completely stopped it at 1 to 800. The amino acids we used were of as high purity as could be obtained. However, some of them doubtless contained impurities. If the impurities were responsible for the injury observed with hydroxyproline, for example, they must be active in extremely small amounts.

The casein hydrolysate prepared from amino acids was less beneficial than the natural product when the two were compared on an equal weight basis. Why should this be? There are two groups of explanations to be considered. One assumes that there is something beneficial in the natural casein hydrolysate which is not present in the synthetic preparation. This can not be eliminated; the natural hydrolysate contains many substances other than amino acids. However, we were not successful in of serine, into that of cystine; phenylalanine, into tyrosine; ornithine, into cystine, proline or glutamic acid. Most of the glutamic acid in the tissue proteins of animals appears to be newly formed. There are, however, limits to these changes in the animal organism. The "indispensable" amino acids are not constructed in the animal body and differences in the power to transform some of the "dispensable" amino acids exist between different kinds of animals, even in the same animal at different ages.

obtaining evidence for any such special beneficial substances.¹⁴ The other possibility is that there is something detrimental in the synthetic hydrolysate which is not active in the natural product. This at first sight appears to be the more probable of the two possible explanations since we found some amino acids to be injurious. On the other hand, these same amino acids occur in the natural casein hydrolysate. The situation is puzzling and requires further investigation. Gladstone (1939) has described interesting antagonisms between amino acids in the nutrition of *B. anthracis*.

In any event, other observations in this laboratory lead us to believe that the beneficial action of vitamin-free casein hydrolysate and other protein hydrolysates is a common phenomenon among the fungi. This means, if our explanation for *T. mentagrophytes* is correct, that many of the fungi are not able to make from inorganic nitrogen, or from a single organic nitrogenous source, the various amino acids needed for their protoplasmic proteins as rapidly as they can be used if furnished preformed in the culture medium.

The development of pleomorphic forms by *T. mentagrophytes* is a complicating circumstance in the study of this organism, but an intriguing one also. The change by this fungus from a slowly growing to a rapidly growing condition, which persists through indefinite subcultures, bears certain analogies to that which occurs when a more or less static animal cell becomes a rapidly growing cancer cell. In both instances the situation is characterized by a greater ability to grow—a greater ability to make cell substance. We do not mean to imply that the same thing happens in both examples; that is beyond the evidence at hand. However, there is a similarity (which may be superficial) between what happens in the development of pleomorphic forms of *T. mentagrophytes* (and in saltations or sectional mutations and similar phenomena in other fungi) and in the development of cancer tissue; both cancer cells and pleomorphic forms are fundamentally growth problems. An elucidation of the physiology of any one of these phenomena may throw light on another.

So far as *T. mentagrophytes* is concerned the pleomorphic forms seem to be characterized by a greater ability to transform ammonium salts and asparagine into the amino acids necessary for protoplasmic protein.¹⁵ These transformations are probably enzymatic, which means that in becoming

¹⁴ Unpublished results, generously transmitted to us by Dr. David R. Goddard, suggest that *Trichophyton interdigitale* (*T. mentagrophytes* (Emmons, 1934)) uses sulfate sulfur far less readily than some forms of organic sulfur. The greater benefit from casein hydrolysate, as compared with the synthetic product prepared from amino acids, may be accounted for by the presence of more organic sulfur in the natural product. We did not, however, observe any marked effect of the amino acids which contain sulfur as compared to those which do not.

¹⁵ The development by the pleomorphic forms of ability to use ammonia resembles to some extent the adaptation of some bacteria to utilize ammonia (Knight, 1932).

pleomorphic the fungus has developed new or more effective enzyme systems than exist in the normal form. Furthermore, it seems probable that more than one enzyme system may be involved. This is suggested by the observation that many pleomorphic strains differing in growth rate on the basal asparagine medium can be isolated, and by the effect of extracts made from the normal and pleomorphic forms on the growth of the same strains. It will be remembered that extracts from the strain which grew most rapidly increased its own growth and that of strains which grew less rapidly. On the other hand, extracts of the slowly growing strains did not affect the growth of more rapidly growing strains. This indicates that the slowly growing strains do not make some of the substances needed by the rapidly growing strains and, contrariwise, the rapidly growing strains produce essential metabolites deficient in those with slower growth rates. Since this seemed to hold for three strains in which the extract of (N) affected only (N), the extract of (B) affected (B) and (N), and the extract of (A) affected (A), (B) and (N), more than one substance and, therefore, more than one enzyme system appears to be concerned.

Of course we cannot say categorically what the growth-promoting materials in these extracts were. It is natural to suggest the amino acids, but it is possible to conceive of other compounds, even unidentified coenzymes associated with the enzyme systems involved in the transformation of one organic nitrogenous compound into another.

Even if the development of pleomorphic forms of *T. mentagrophytes* is fundamentally a change in the ability of the organism to transform a single source of nitrogen into the various amino acids required for the construction of cell substance, the same explanation may not apply to other similar phenomena in the fungi. Thus mutant forms of *Fusarium avenaceum* developed the power to synthesize biotin which the "normal" form lacked (Robbins and Ma, 1941).

This investigation emphasizes the concept of growth as depending upon essential metabolites, a complete or partial deficiency of any one of which may become a limiting factor. Such limiting essential metabolites may be vitamins or their precursors, one or more amino acids, or any other compound in the chains which result in the formation of cell substance.

SUMMARY

Trichophyton mentagrophytes (*T. gypseum*) isolated from a human subject was maintained in a freely sporulating condition by frequent transfers. This normal form was unable to utilize ammonium nitrate and grew much more rapidly with peptone, casein hydrolysate, gelatine hydrolysate, and other natural products than with asparagine. The fungus appeared to be autotrophic as far as vitamins were concerned. The beneficial action of peptone, casein hydrolysate and other protein hydrolysates is be-

lieved to be caused by amino acids. However, although mixtures of amino acids were beneficial none was found as satisfactory as casein hydrolysate. The fungus used asparagine or any one of 14 amino acids as a source of nitrogen, but no single compound was as satisfactory as a suitable mixture of amino acids. No evidence for indispensable amino acids was obtained. It is suggested that the superiority of a mixture of amino acids depends upon the inability of the fungus to transform inorganic nitrogen or a single organic nitrogenous compound into the various amino acids needed for protoplasmic protein as rapidly as they can be used if furnished preformed in the culture medium. An approximation of casein hydrolysate prepared from amino acids was not as satisfactory as the natural casein hydrolysate. Fractions or extracts obtained with ethyl, butyl or octyl alcohol from natural casein hydrolysate did not account for its greater effectiveness. Some amino acids, especially hydroxyproline, were injurious.

Mutant or pleomorphic forms were isolated and maintained in culture. The development of pleomorphic forms was delayed by incubation at lower temperatures. Reversion from pleomorphic to normal was not observed. The pleomorphic forms utilized ammonia and asparagine more readily than did the normal form. Extracts of a pleomorphic form favorably influenced its own growth and that of strains less vigorous. It is suggested that pleomorphism of *T. mentagrophytes* involves the development of a better mechanism for transforming ammonia or a single organic nitrogenous source into cell substance.

NEW YORK BOTANICAL GARDEN AND
DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY

LITERATURE CITED

- ALMQUIST, H. J. 1944. The role of proteins in nutrition. The chemistry of the amino acids and proteins with addendum. Edited by C. L. A. Schmidt. 2nd Ed. BRUMPT, E. 1927. *Precis de parasitologie*. Quatrième Edition. Masson et Cie. Paris.
- BUTLER, E. T., W. J. ROBBINS, AND B. O. DODGE. 1941. Biotin and the growth of *Neurospora*. *Science* 94: 262-263.
- EMMONS, C. W. 1932. Pleomorphism and variation in the Dermatophytes. *Archiv. Dermat. and Syph.* 25: 987-1001.
- . 1934. Dermatophytes; a natural grouping based on the form of the spores and accessory organs. *Archiv. Dermat. and Syph.* 30: 337-362.
- GLADSTONE, G. P. 1939. Inter-relationships between amino-acids in the nutrition of *B. anthracis*. *Brit. Jour. Exp. Path.* 20: 180-200.
- KNIGHT, B. C. J. G. 1936. Bacterial nutrition. Material for a comparative physiology of bacteria. Great Britain. Med. Res. Council Spec. Rpt. Ser. 210. 182 pp.
- ROBBINS, WILLIAM J., AND K. C. HAMNER. 1940. Effect of potato extracts on growth of *Phycomyces*. *Bot. Gaz.* 101: 912-927.

- , AND F. KAVANAGH. 1942. Hypoxanthine, a growth substance for *Phycomyces*. Proc. Nat. Acad. Sci. U.S.A. 28: 65-69.
- , AND ROBERTA MA. 1941. Biotin and the growth of *Fusarium avenaceum*. Bull. Torrey Bot. Club 68: 446-462.
- . 1942A. Vitamin deficiencies of *Ceratostomella*. Bull. Torrey Bot. Club 69: 184-203.
- . 1942B. Vitamin deficiencies of *Trichophyton discoides*. Bull. Torrey Bot. Club 69: 509-521.
- . 1944. A *Rhodotorula* deficient for para-aminobenzoic acid. Science 100: 85-86.
- SCHOPFER, W. H., AND S. BLUMER. 1942. Recherches sur le besoin en facteurs de croissance vitaminiques et le pouvoir de synthèse d'un "Trichophyton." Le problème du conditionnement des pouvoirs de synthèse. Compt. Rend. Soc. d phys. et d'histoire naturelle de Geneve 59: 106-112.
- STEINBERG, R. A. 1942. Effect of trace elements on growth of *Aspergillus niger* with amino acids. Jour. Agric. Res. 64: 455-472.
- . 1944. Variants in fungi: formation, reversion and prevention. Science 100: 10.
- , AND CHARLES THOM. 1942. Reversions in morphology of nitrite-induced "mutants" of *Aspergillus* grown on amino acids. Jour. Agric. Res. 64: 645-652.

EFFECTS OF DEFICIENCIES OF CERTAIN MINERAL ELEMENTS ON THE DEVELOPMENT OF TARAXACUM KOK-SAGHYZ¹

Bernard S. Meyer

THE RUSSIAN dandelion, *Taraxacum kok-saghyz* Rodin, was discovered in 1931 on the high plateau of Tian-Shan in Kazakhstan, Russia. The potentialities of this plant as a source of rubber were recognized from the first and it apparently has been grown on a fairly extensive scale as a crop plant in some parts of Russia, especially the Ukraine. Considerable progress also appears to have been made in that country in the selection of high rubber content strains of the plant and in its agronomic management as a crop. As an outcome of the war emergency, seeds of this plant were first brought to the United States in May, 1942 (Brandes, 1942), and it has been the subject of a number of investigations in this country since that time. Warmke (1943, 1944) has published on the macrosporangogenesis, fertilization, and embryology of this plant, Artschwager and McGuire (1943) on its morphology and anatomy, Borthwick, Parker, and Scully (1943) on its reactions to temperature and photoperiod, Marth and Hamner (1943) on the effect of growth substances on its vegetative propagation, and Levitt and Hamm (1943) on a method of increasing the rate of seed germination of this species. To date, however, no papers have been published which report the results of critical investigations of the mineral nutrition of this species. In this paper the results of an investigation of some of the effects of deficiencies of nitrogen, phosphorus, calcium, magnesium, and potassium on the development of kok-saghyz plants are reported.

EXPERIMENTAL METHODS.—Two-gallon glazed porcelain pots² were used as the containers in all

¹ Received for publication April 4, 1945.

Papers from the Department of Botany, the Ohio State University, No. 480. A part of the work reported in this paper was done while the author was employed as Associate Physiologist in Rubber Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture. Another part of this investigation was aided by a research grant from the Ohio Academy of Science. The substantial assistance of Dr. H. T. Scofield with one of the series of experiments is also gratefully acknowledged.

² Sold by the Red Wing Potteries, Chicago, Ill., as "urn liners."

experiments. Six pots were used in each experiment and the experiment was repeated five times. The drain hole in the bottom of each pot was plugged with a rubber stopper through which was inserted a glass drain tube 8 mm. in diameter. A square of glass wool, about 5 × 5 cm., was laid across the top of the stopper, which was flush with the bottom of the pot. Each pot was filled to within about 5 cm. of the top with a coarse (#3½) silica sand (essentially a fine gravel) on top of which was placed a layer of finer (#5½) silica sand about 4 cm. deep.³

The sand in each pot was flushed several times with tap water, about one hundred untreated seeds of kok-saghyz were sprinkled over its surface, and a very thin layer of #5½ sand drifted over the layer of seeds. The seeds used in the first two experiments were of the 1941 crop imported directly from Russia; those used in the last three experiments were of the 1942 crop, harvested at St. Paul, Minnesota. Except during the summer months of high atmospheric vapor pressures, the pots were kept covered with glass plates for two to three weeks after sowing the seeds. The young seedlings were protected from direct sunlight with a layer of cheesecloth. Germination of most seeds occurred in two to four days and the percentage of germination, although not determined, was obviously high.

The rosettes of kok-saghyz plants develop very slowly during the first six to eight weeks. During this period the stand of seedlings was gradually thinned until ultimately only three plants were left in each pot (in experiment 1 only two plants were left per pot). During the thinning process weaker plants were gradually eliminated. Also non-kok-saghyz dandelions, present in considerable numbers because of seed contamination but readily identifiable even as young seedlings, were removed. The thinning was done in such a way as to provide a suitable spacing for the plants which remained in each pot.

³ The #3½ and 5½ sands were obtained from the Industrial Silica Corporation, Youngstown, Ohio.

TABLE 1. *Composition of solutions. Milliliters of 1 M single salt solution to make 3 liters of nutrient solution. To each 3 liters of the completed solution are also added 3 ml. of ferric tartrate solution and 3 ml. of the minor element solution.*

Solution	KNO ₃	KH ₂ PO ₄	Ca(NO ₃) ₂	MgSO ₄	KCl	CaCl ₂	Na ₂ SO ₄	NaH ₂ PO ₄	NaNO ₃	Mg(NO ₃) ₂
Complete	6	6	9	6
Minus N	..	6	..	6	6	9
Minus P	6	..	9	6	6
Minus Ca	6	6	6	9
Minus Mg	6	6	9	6
Minus K	9	6	6	6	..

In all experiments the young seedlings were watered with tap water applied as a fine spray for approximately the first two weeks of their existence. During the next two weeks they received in addition an occasional spraying with half-strength complete solution (table 1). When the plants were about one month old, irrigation with a full-strength complete solution was started. After about two weeks on this solution the pots were thoroughly flushed with distilled water and from then on each pot was irrigated with a different one of the six solutions used (table 1). Because of the small size and slow rate of development of the seedlings it was found necessary to allow them to develop for a short time with a full regimen of mineral elements before subjecting them to conditions of acute mineral element deficiency. The set of solutions used has previously been employed in this laboratory with entirely satisfactory results in demonstrating mineral deficiency symptoms in several species of plants.

The ferric tartrate solution used in making the nutrient solutions was prepared by adding 5 g. of ferric chloride and 5 g. of tartaric acid per liter to distilled water. The minor element solution had the following composition: 2.5 g. H₃BO₃, 1.8 g. MnCl₂·4H₂O, 0.1 g. ZnCl₂, 0.05 g. CuCl₂·2H₂O, and 0.075 g. MoO₃ per liter of distilled water.

The calculated osmotic pressures of all of the solutions in this series lie within the range of 0.50 ± 0.03 atm. The initial pH values of all the solutions (glass electrode) lie within the range of 3.8–4.2. As used in these experiments the pH values of all of these solutions showed a gradual drift towards higher values, the magnitude of the drift varying considerably, depending upon the particular solution, the size and stage of development of the root system, and the prevailing climatic conditions. Values in excess of pH 7.5 were never attained, however, in any of the solutions during the course of this investigation.

The pots stood on racks or benches so constructed that a one-gallon amber reservoir bottle could stand under each pot. The drain tube from the pot was connected, by means of a short length of rubber tubing, with a glass tube which extended through the stopper of the corresponding reservoir bottle nearly to its bottom. Three liters of the appropriate solution were placed in each bottle. Irrigation was accomplished by allowing a stream of

air from a compressed air line to flow into the reservoir bottle through an inlet tube in the stopper. The solution in the reservoir was displaced upwards until the sand in the pot was saturated to its surface; after disconnecting the air line the solution slowly drained back into the reservoir. Most commonly a schedule of two irrigations per day—one in the late morning and one in the late afternoon—was followed. During periods of hot summer weather, however, three irrigations were often given per day, and during periods of dull days in the winter one irrigation per day was often found to be sufficient. Before each irrigation the volume of solution in each reservoir was restored to three liters by adding distilled water. Since the sand retained about 500 ml. of solution after drainage, there were always in the system of pot plus reservoir bottle about 3.5 liters of solution. As a general rule the solutions in the reservoirs were discarded and replaced by entirely new solutions approximately every two weeks. During periods of rapid growth, replacement of old solutions with fresh ones was often made at more frequent intervals, and during periods of very slow growth the interval between solution replacements was sometimes lengthened to as much as three weeks.

Generally speaking the central Ohio summer climate is too warm for the best development of kok-saghyz plants, and under greenhouse conditions in summer the rosettes show a progressive retrogression in size. Hence from about the first of June to about the middle of October the cultures were kept outdoors. High temperature effects were partially ameliorated by shading with a single layer of cheesecloth supported on a frame several feet above the plants and also by so placing the cultures that the plants were not exposed to direct insolation until mid-morning. By thus cutting the number of hours per day that the plants were exposed to direct sunlight and also by reducing the intensity of the incident light, excellent development of the plants was obtained even during the hottest months. One complication of working with cultures of this type under outdoor conditions is that surplus water accumulates in the reservoir during each rain. However, because of the rapid loss of water by transpiration and evaporation during the summer, this never resulted in more than a temporary dilution of the

TABLE 2. Fresh weights and dry weights in grams, and percentage rubber contents on a dry weight basis of the roots of kok-saghyz plants grown in complete solutions and in solutions deficient in certain essential mineral elements.

Exper. number	Duration of experiment	Complete			N deficient			P deficient			Ca deficient			Mg deficient			K deficient		
		Fresh wt. roots	Dry wt. roots	Rub-ber %	Fresh wt. roots	Dry wt. roots	Rub-ber %	Fresh wt. roots	Dry wt. roots	Rub-ber %	Fresh wt. roots	Dry wt. roots	Rub-ber %	Fresh wt. roots	Dry wt. roots	Rub-ber %	Fresh wt. roots	Dry wt. roots	Rub-ber %
1	4/7/43-9/7/43	37.0	6.53	1.5	4.3	1.15	0.5	33.3	6.15	0.6	39.3	6.96	0.6	28.3	5.34	0.2	24.1	3.35	1.0
2	5/6/43-10/10/43	45.7	10.30	2.0	7.8	2.02	2.2	51.1	12.09	1.7	41.5	8.23	1.8	14.2	3.45	1.6	15.5	3.58	1.6
3	7/26/43-1/19/44	11.6	2.10	1.6	5.2	1.06	1.4	14.2	2.78	2.9	9.0	1.73	3.0	7.6	1.46	1.6	8.4	1.62	1.7
4	2/10/44-7/2/44	20.9	4.54	0.75	12.8	3.40	1.35	25.6	5.59	1.65	18.7	4.26	0.75	13.8	3.08	0.6	18.6	4.30	0.3
5	5/23/44-11/4/44	22.7	4.48	1.65	2.4	0.44	0.45	13.6	2.96	1.20	14.7	2.44	2.25	9.1	1.75	1.35	12.5	2.29	1.65
Total weight		137.9	27.95		32.5	8.07		137.8	29.57		123.2	23.62		73.0	15.08		79.1	15.14	
Average weight per plant		9.9	2.00		2.3	0.58		9.9	2.11		8.8	1.69		5.2	1.08		6.1	1.16	
Average per cent rubber				1.5			1.2			1.6			1.7			1.1			1.2

solution. Even after a heavy thunderstorm the water in the reservoirs usually dropped to its normal level in two or three days. Prior to the first of June and after the middle of October the pot cultures were kept in a greenhouse, in which the attainment of excessive temperatures on bright days was avoided as far as feasible by appropriate ventilation.

The relative position of the individual pots in each experiment was shifted according to a randomizing scheme each time the solutions were changed in order to minimize the possible effects of an asymmetric distribution of light or any other environmental factor. All dead basal leaves of the rosettes were removed from the plants from time to time.

As grown under the conditions of these experiments, kok-saghyz plants were quite susceptible to infestations of a species of thrips (*Thrips nigropilosus*) which at times caused some minor injury. Moderately good control of the thrips was attained by painting the leaves of infested plants with a rotenone emulsion at appropriate intervals.

The duration of each experiment from the time of sowing the seed to the time of harvesting the roots was from five to six months, this being the approximate length of the growing season when kok-saghyz is grown as an annual crop. Upon dismantling each experiment, all leaves were plucked from the plants. The entire root system of each plant plus the short stem constituted the plant material used in all determinations. Plants from which the leaves had been stripped are henceforth designated simply as "roots." The fresh weight of each individual root was determined immediately and, after drying for about 48 hours at 70°C., determinations were also made of the dry weights of the individual roots. All root samples were also analyzed for their percentage rubber content.⁴

RESULTS AND DISCUSSION.—Data on the yield of roots in terms of both fresh and dry weights and on percentage rubber contents of the roots for the five experiments are summarized in table 2. All fresh and dry weight values except totals and averages as given in this table are aggregate values for all plants in each pot. Two plants were left in each pot during the first experiment and three plants were left per pot in each of the other experiments. Not a single one of the experimental plants was lost from disease or any other cause during the course of the investigation. However, one plant in the minus potassium pot of experiment 2 turned out to be a non-kok-saghyz dandelion, but was not so recognized until rather late in the experiment, hence only two plants were left in this pot. The only other irregularity occurred in the minus phosphorus pot of experiment 5. The original of this pot was accidentally destroyed two weeks after the beginning of

⁴ Rubber analyses were made at the U. S. D. A. Rubber Plant Field Laboratory, St. Paul, Minnesota, or at the Laboratory of Rubber Investigations, at the Plant Industry Station, Beltsville, Maryland, by a turbidimetric method which has not been published.

the experiment and was replaced at that time by another. The plants in this pot were therefore two weeks younger at the time of harvest than the plants in other pots in this experiment.

The root yields for the plants receiving all essential ions were very different from one experiment to the next, doubtless reflecting the different patterns of environmental conditions which prevailed during the different experiments. In spite of the markedly different yields in the different experiments it is noteworthy that the trend of the results is very similar in all of them and substantially the same conclusions regarding effects of deficiencies of the several ions on the development of roots would be drawn from any single one of the experiments as from an overall consideration of the experiments as a group.

In evaluating the results presented in this table it should be recalled that all plants received a complete solution for about one month before irrigation of all cultures, except the checks, was begun with solutions deficient in one of the essential ions. The proportionate concentrations of the several ions in the complete solution should therefore be considered in interpreting the results. As shown in table 1, the various ions were present in the complete solution in the following proportions: $8 \text{ NO}_3^- : 4 \text{ K}^+ : 3 \text{ Ca}^{++} : 2 \text{ H}_2\text{PO}_4^- : 2 \text{ Mg}^{++}$.

As shown by the data in table 2, deficiency of nitrogen resulted in a smaller yield of roots in every one of the experiments than deficiency of any of the other elements studied.

The root development of both magnesium- and potassium-deficient plants was also markedly less than for the plants receiving the complete solution, fresh and dry weight yields of the roots being roughly only half as great as for the check plants. In four of the five experiments the root yield of magnesium-deficient plants was the lowest and in the other experiment second lowest except for the nitrogen-deficient plants. In three of the five experiments the root yield of potassium-deficient plants was the lowest next to yields of nitrogen- and magnesium-deficient plants; in one experiment the root yield of such plants was second lowest; and in one experiment the fourth lowest.

The average values for the root yields of magnesium-deficient plants are not significantly different from those for potassium-deficient plants. There are reasons for believing that the potassium requirement of kok-saghyz may be greater than the magnesium requirement or at least more nearly equal to it than superficial inspection of these data would suggest. The concentration of potassium ions in the complete solution which all plants received for about one month while still in the seedling stage was twice as great as the concentration of magnesium ions. In all probability, therefore, the young plants absorbed greater numbers of potassium ions than of magnesium ions before being placed on a deficiency regime. In spite of this probability reduction in root growth is almost as great for potas-

sium-deficient plants as for magnesium-deficient plants. It should also be noted that the potassium-deficient plants were grown in a solution in which sodium ions were available (table 1). According to some workers (Mullison and Mullison, 1942) sodium can in part replace potassium in the mineral nutrition of at least some species of plants. The possibility at least must therefore be entertained that the presence of available sodium ions may have in part ameliorated the deficiency of potassium in the plants grown in cultures lacking this latter element. Although it is obvious from the data that both the magnesium and potassium requirements of kok-saghyz plants are relatively high, it is not possible to state with certainty which of these elements is necessary in the greater quantity. The relatively high magnesium requirement for normal root development of kok-saghyz plants is in itself, however, a noteworthy fact.

The average dry weight and average fresh weight for the roots of calcium-deficient plants were not greatly less than for the check plants, in spite of the fact that some of the calcium-deficient plants exhibited foliar symptoms of calcium deficiency as described later. In other words the "calcium-deficient" plants were able to absorb calcium ions in sufficient quantities during the few weeks the roots had access to a complete solution to meet almost the entire quota of this element needed during a six months' growth period. A clear inference from this finding is that the calcium requirement of kok-saghyz is relatively low.

The yield of roots from the plants receiving a phosphorus-deficient solution was, on the average, about the same as that for the plants receiving the complete solution. Apparently the so-called phosphorus-deficient plants were able to absorb phosphate ions in sufficient quantities during the few weeks the plants had access to a complete solution to meet the needs of the plants for this element during the entire growth period. The most probable inference from these results is that the requirement of kok-saghyz plants for phosphate ions is less than for any of the other four kinds of elements investigated. The fact that the proportion of phosphate ions in the complete solution from which the plants must have obtained all of the phosphorus used throughout their growth cycle was lower than for any other except magnesium supports this conclusion.

It should also be recalled that the "phosphorus-deficient" plants in experiment 5 had a two weeks' shorter growth period than the other plants in this experiment. For this reason the values for average root yields of the plants receiving a solution lacking in phosphorus are probably slightly smaller than they would otherwise have been. In three of the five experiments the fresh weight and dry weight yield from "phosphorus-deficient" plants actually exceeded that from plants in the complete solution. This suggests the possibility that the concentration of phosphates in the complete solution,

even though relatively low, may have been slightly too high for the best development of the roots of this plant.

The percentage rubber contents (table 2) for all of the kok-saghyz roots grown in these experiments are relatively low compared with many other reported values, and the range of values is also small. The probable explanation of this fact is that certain climatic conditions, most likely temperature and light intensity, were not the most favorable during the course of these experiments for the synthesis and accumulation of rubber. Temperature (Bonner, 1943) and light intensity (Mitchell *et al.*, 1944) have been shown to have a signal influence on the accumulation of rubber in the guayule (*Parthenium argentatum* Gray), another species of rubber-bearing composite, and there are numerous indications that this is also true for kok-saghyz. From table 2 the average percentage rubber content appears to be lower for the magnesium-deficient, nitrogen-deficient, and potassium-deficient plants than for the calcium- or phosphorus-deficient plants or for those which were rooted in a complete solution. The same mineral element deficiencies which resulted in the greatest reduction in root yield appear, therefore, to have the greatest effect in reducing the percentage rubber content of the roots. However, the statistical probability that even the greatest differences shown in average percentage rubber content from one treatment to the next are significant is small. These results regarding the effects of mineral elements upon the percentage rubber content of kok-saghyz roots cannot be accepted as conclusive, therefore, but only as indicating possible trends.

The total yield of rubber in this plant depends both on the dry weight yield and percentage rubber content of the roots. The results of the present investigation indicate that mineral salts almost certainly influence the yield of rubber in kok-saghyz more through their effect on the gross yield of the roots than through effects on the synthesis of rubber.

It is of interest to compare the results of this investigation with those of Bonner (1944) on the effects of mineral elements on growth and rubber accumulation in guayule. Both growth and rubber accumulation in guayule were retarded by deficiencies of nitrogen and magnesium; this was clearly true for growth in kok-saghyz and is probably also true for rubber accumulation. The main differences between the two species appear to be that the phosphorus requirement for normal development is somewhat greater in guayule than in kok-saghyz, while the opposite is true of the potassium requirement.

An attempt was made to discover readily identifiable foliar symptoms of mineral element deficiencies in kok-saghyz. Because of its rosette habit of growth, however, recognition of such deficiency symptoms is more difficult in this species than in many others. Differential development of chlorosis between younger and older leaves, which can often

be recognized as a deficiency symptom in many other species, is not readily observable in kok-saghyz plants because the older, lower leaves of the rosette usually die off before any such effects become apparent. Specific symptoms of diagnostic value have been recognized only for nitrogen and calcium deficiencies. Nitrogen-deficiency symptoms become evident promptly after the supply of nitrates to the roots is curtailed. The developing leaves fail to expand and fail to elongate fully, shortly become a grayish green in color, and often develop a reddish pigmentation along the midrib. Ultimately the leaves fade to a yellow color and die shortly thereafter. Subsequently other undersized leaves may develop from the crown of a nitrogen-deficient plant, which in turn go through the same cycle. Calcium deficiency was evidenced in most, but not all, plants deficient in this element by a necrosis of the leaf tips of the younger leaves. Under the conditions of these experiments, at least, this symptom usually did not appear until rather late in the growth cycle.

The only foliar symptom of a deficiency of any of the other three elements studied was a retrogression in the size of the rosettes which often did not start until rather late in the growth cycle. This occurs as a result of the gradual dying off of the larger, older leaves, while each new cycle of developing leaves elongates and expands less than the preceding one. Ultimately the rosette may entirely disappear. This retrogression in rosette size was shown by almost all of the nitrogen-, potassium-, and magnesium-deficient plants and by an occasional phosphorus- or calcium-deficient plant. Obviously such a growth reaction is not diagnostic for any particular element and in fact may be induced not only by mineral element deficiencies but also by unfavorable environmental conditions such as low light intensity or high temperature.

No marked correlations were observed between floral initiation and mineral element deficiency in this species. Several flowers often developed on stunted, moribund nitrogen-deficient plants, for example, while lush, healthy plants receiving a complete solution remained flowerless. The proportion of plants flowering in any one experiment varied greatly with the season and in no experiment did all of the plants flower. Climatic conditions, especially temperature, day length and probably light intensity, have much more influence on the initiation of flowers in this species than the proportions of mineral elements available. A far larger proportion of the plants (67 per cent) flowered in experiment 3 than in any of the others. This is in accord with the finding of Warmke (1944) that the peaks of flowering activity in this species occur in November and April. In none of the present experiments were the plants beyond the young seedling stage in April, and experiment 3 was the only one carried through the month of November. Borthwick *et al.* (1943) concluded that a cool temperature and a long photoperiod are most favorable for early

blooming of the seedlings of kok-saghyz. The flowering behavior of the plants in the present investigation is consistent with this conclusion if the predominant role is ascribed to cool temperature.

SUMMARY

An investigation was made of the effects of deficiencies of nitrogen, phosphorus, calcium, magnesium, and potassium on the growth and rubber accumulation in *Taraxacum kok-saghyz* Rodin. Deficiency of nitrogen resulted in by far the smallest fresh or dry weight yield of roots of any of the treatments. Deficiency of either magnesium or potassium also resulted in appreciably smaller fresh and dry weight yields of roots as compared with

the checks while deficiency of calcium or phosphorus had relatively much less effect. There are indications that the same mineral element deficiencies which have the most marked retarding effects on root development also have the greatest influence in reducing rubber accumulation in the roots, but the evidence on this point is not conclusive. Specific foliar symptoms were recognized only for nitrogen and calcium deficiencies. No marked correlations were found between floral initiation and mineral element deficiency.

BOTANY DEPARTMENT,
OHIO STATE UNIVERSITY,
COLUMBUS, OHIO

LITERATURE CITED

- ARTSCHWAGER, E., AND RUTH C. MCGUIRE. 1943. Contribution to the morphology and anatomy of the Russian dandelion (*Taraxacum kok-saghyz*). U. S. Dept. Agric. Tech. Bull. 843.
- BONNER, J. 1943. Effects of temperature on rubber accumulation by the guayule plant. Bot. Gaz. 105: 233-243.
- . 1944. Effect of varying nutritional treatments on growth and rubber accumulation in guayule. Bot. Gaz. 105: 352-364.
- BORTHWICK, H. A., M. W. PARKER, AND N. J. SCULLY. 1943. Effects of photoperiod and temperature on growth and development of kok-saghyz. Bot. Gaz. 105: 100-107.
- BRANDES, E. W. 1942. Rubber from the Russian dandelion. Agriculture in the Americas 2: 127-131.
- LEVITT, J., AND P. C. HAMM. 1943. A method of increasing the rate of seed germination of *Taraxacum kok-saghyz*. Plant Physiol. 18: 288-293.
- MARTH, P. O., AND C. L. HAMNER. 1943. Vegetative propagation of *Taraxacum kok-saghyz* with the aid of growth substances. Bot. Gaz. 105: 35-48.
- MITCHELL, J. W., A. GERALDINE WHITING, AND H. M. BENEDICT. 1944. Effect of light intensity and nutrient supply on growth and production of rubber and seeds by guayule. Bot. Gaz. 106: 83-95.
- MULLISON, W. R., AND ETHEL MULLISON. 1942. Growth responses of barley seedlings in relation to potassium and sodium nutrition. Plant Physiol. 17: 632-644.
- WARMKE, H. E. 1943. Macrosporogenesis, fertilization, and early embryology of *Taraxacum kok-saghyz*. Bull. Torrey Bot. Club 70: 164-173.
- . 1944. Self-fertilization in the Russian dandelion, *Taraxacum kok-saghyz*. Amer. Nat. 78: 285-288.

INFLUENCE OF THE PROPORTIONS OF KH_2PO_4 , MgSO_4 , AND NaNO_3 IN THE NUTRIENT SOLUTION ON THE PRODUCTION OF PENICILLIN IN SURFACE CULTURES¹

Robertson Pratt

THE IMPORTANCE of penicillin has stimulated interest in studies of cultural conditions suited to increase its biosynthesis. Much effort has been directed toward study of organic substrata that may be utilized by species of *Penicillium* for synthesis of penicillin in different types of media but factors of inorganic nutrition seem to have received relatively scant attention, especially in surface cultures.

Abraham *et al.* (1941) observed that maximum production of penicillin occurred when the pH of the fermentation medium was about 7.0. Later work suggested again the desirability of maintaining the pH of the culture below 7.5 because of the greater stability of penicillin in aqueous solutions in this range (Abraham and Chain, 1942). Subsequently,

¹ Received for publication April 16, 1945.

Currently on leave from the University of California College of Pharmacy.

Acknowledgment is made of the capable technical assistance in the laboratory of Elizabeth Arnold, Dorothy Leif, Iola Semas, and Patricia Streater.

attempts were made to maintain a favorable pH in the fermentation medium by use of appropriate phosphate buffers (Challinor and MacNaughton, 1943) and improved production of penicillin was reported when the proper combination of M/15 KH_2PO_4 and M/15 Na_2HPO_4 was incorporated in the medium. Dimond and Peltier (1945) reported that the pH of the nutrient solution may be maintained at a virtually constant level throughout the growth period of *Penicillium notatum* by selection of the proper combination of carbohydrate and inorganic and organic sources of nitrogen. These investigations, however, were not concerned primarily with the relation between inorganic nutrients and penicillin production.

The present study was undertaken to ascertain the influence, if any, of the proportions of the inorganic salts in the culture medium on the accumulation of penicillin in cultures of *Penicillium notatum*. When the work was begun, it was believed that this type of experimentation might prove fruitful be-

TABLE 1. Composition of the solutions employed for studying the accumulation of penicillin in surface cultures of *Penicillium notatum*. The initial total molarity of the three salts was 0.04 in each solution. The molecular proportions are expressed as percentages of this value. The numbers of the solutions correspond to the numbers within the triangle in figure 1.

Solution number	Molecular proportions ^a			Solution number	Molecular proportions ^a		
	KH ₂ PO ₄	MgSO ₄	NaNO ₃		KH ₂ PO ₄	MgSO ₄	NaNO ₃
1	96.0	2.0	2.0	34	20.0	10.0	70.0
2	90.0	5.0	5.0	35	20.0	20.0	60.0
3	80.0	2.5	17.5	36	20.0	30.0	50.0
4	80.0	10.0	10.0	37	20.0	40.0	40.0
5	80.0	17.5	2.5	38	20.0	50.0	30.0
6	67.5	5.0	27.5	39	20.0	60.0	20.0
7	70.0	10.0	20.0	40	20.0	70.0	10.0
8	70.0	20.0	10.0	41	20.0	77.5	2.5
9	67.5	27.5	5.0	42	9.0	2.5	88.5
10	60.0	2.5	37.5	43	10.0	10.0	80.0
11	60.0	20.0	20.0	44	7.5	22.5	70.0
12	60.0	37.5	2.5	45	10.0	30.0	60.0
13	55.0	10.0	35.0	46	7.5	35.0	57.5
14	55.0	35.0	10.0	47	10.0	40.0	50.0
15	50.0	20.0	30.0	48	7.5	47.5	45.0
16	50.0	30.0	20.0	49	10.0	50.0	40.0
17	47.5	5.0	47.5	50	7.5	60.0	32.5
18	47.5	47.5	5.0	51	10.0	70.0	20.0
19	40.0	2.5	57.5	52	10.0	80.0	10.0
20	40.0	10.0	50.0	53	2.0	2.0	96.0
21	40.0	20.0	40.0	54	5.0	5.0	90.0
22	40.0	30.0	30.0	55	2.5	12.5	85.0
23	40.0	40.0	20.0	56	2.5	20.0	77.5
24	40.0	50.0	10.0	57	2.5	32.5	65.0
25	40.0	57.5	2.5	58	2.5	45.0	52.5
26	35.0	2.5	62.5	59	1.0	55.0	44.0
27	30.0	10.0	60.0	60	2.5	57.5	40.0
28	30.0	20.0	50.0	61	2.5	70.0	27.5
29	30.0	30.0	40.0	62	5.0	77.5	17.5
30	30.0	40.0	30.0	63	2.5	87.5	10.0
31	30.0	50.0	20.0	64	5.0	90.0	5.0
32	30.0	60.0	10.0	65	2.0	96.0	2.0
33	20.0	2.5	77.5				

^a Each solution contained in addition to the three salts shown 0.111 M. lactose, 0.00015 M. ZnSO₄·7H₂O, 0.00183 M. Phenyl acetic acid, and corn steep liquor solids 40 gm. per liter.

cause examination of the formula of the then currently employed culture solution indicated enormous disparity of certain ions in proportion to others when compared with solutions that have been found to be optimum for the biochemical activities of other plants.

It should be emphasized that the results presented below are not offered as a categorical or definitive answer to the problem that was investigated. They are the outcome of a piece of industrial research designed to answer a question that was critically important when the work was performed—i.e., what can be done *now* to increase production with present materials and with present sources of supply. Use of corn steep liquor from other companies or other slight modifications in the fermentation liquor might have altered the results.² Presentation of the present data is considered important, however, because they illustrate the influ-

² Different lots of steep liquor from the Clinton Company all yielded similar results.

ence that quantitative variations in the inorganic composition of a fermentation medium may exert on mold metabolism and they may, therefore, be useful to academic workers engaged in fundamental investigations.

Qualitatively, all the solutions used in the experiments were similar. Quantitatively, the solutions differed in the proportions of the three salts, KH₂PO₄, MgSO₄·7H₂O, and NaNO₃.

MATERIALS AND METHODS.—*Penicillium notatum*, strain 1249.B4³ was used throughout the experiments. Mushroom spawn bottles (46 ounce) plugged lightly with gauze and cotton and laid on their sides were used as culture vessels. Initially, each culture contained 270 ml. of culture medium, the maximum depth of which was 2.5 cm. The surface/volume ratio was approximately 0.615. The nutrient solution contained lactose, 0.111 M. (40 gm./L.); corn steep liquor solids (Clinton Co.), 40 gm./L.;

³ This strain is a variant of *Penicillium notatum*, NRRL strain 1249.B21.

TABLE 2. Relative potency on successive days in media with different proportions of KH_2PO_4 , $MgSO_4$, and $NaNO_3$.

Solution number	Relative potency on day				Solution number	Relative potency on day			
	4	5	6	7		4	5	6	7
1	0.81	0.88	1.09	0.92	34	0.81	0.96	1.10	0.92
2	>1.00	0.74	1.01	1.08	35	0.76	1.03	1.16	1.08
3	1.08	1.16	0.82	0.67	36	0.52	1.03	0.90	0.87
4	0.67	0.74	1.12	1.14	37	0.78	1.10	0.91	1.25
5	0.94	1.63	0.80	0.76	38	1.14	1.07	0.93	0.91
6	0.86	1.14	1.04	0.79	39	0.77	1.21	1.20	1.05
7	1.36	1.23	1.13	0.90	40	0.64	1.25	1.00	1.64
8	1.19	1.10	1.10	0.98	41	0.86	1.22	0.80	0.62
9	1.02	1.02	0.88	1.14	42 ^a	1.00	1.00	1.00	1.00
10	0.99	1.34	0.99	0.72	43	>1.00	1.03	1.24	1.34
11	0.67	0.98	1.11	1.04	44	1.09	0.93	1.15	0.94
12	0.72	0.97	0.67	0.62	45	1.12	0.93	1.02	0.76
13	0.69	1.33	1.05	0.84	46	1.05	1.06	1.13	0.86
14	0.39	0.34	0.21	0.16	47	0.95	1.04	0.93	0.80
15	0.64	...	1.21	0.95	48	0.72	1.01	1.11	0.91
16	0.76	1.05	1.09	0.78	49	0.81	0.94	0.92	0.93
17	1.12	1.56	1.54	1.29	50	0.88	0.94	0.96	0.73
18	0.95	1.14	1.02	0.74	51	0.59	0.91	0.86	0.74
19	0.80	1.09	1.04	0.87	52	0.87	0.97	0.91	1.18
20	1.14	1.27	1.31	1.12	53	0.83	1.07	1.37	1.14
21	0.69	1.18	0.98	1.17	54	1.00	1.00	1.00	1.00
22	0.83	1.05	0.88	1.24	55	0.95	1.27	1.31	0.99
23	0.89	0.99	0.97	1.08	56	0.87	1.52	0.96	0.97
24	1.00	1.14	0.91	0.82	57	1.12	1.00	0.96	0.84
25	0.83	1.04	0.82	0.65	58	1.09	1.20	1.11	1.10
26	1.10	1.08	1.01	0.98	59	0.79	1.07	1.01	0.95
27	1.12	1.57	0.99	0.91	60	1.05	0.96	0.90	0.86
28	0.64	1.13	1.15	0.74	61	0.91	1.15	0.81	0.81
29	0.96	1.33	0.95	0.94	62	0.69	0.86	0.84	0.71
30	1.17	0.94	1.34	0.80	63	0.88	0.96	0.96	0.74
31	1.17	1.24	1.25	0.72	64	1.09	1.12	0.99	0.85
32	0.78	1.12	0.99	0.83	65	1.06	1.07	1.16	0.89
33	1.10	1.06	1.22	1.13					

^a The absolute values in the standard solution (No. 42) from the second through the eighth day were, respectively, 8, 26, 50, 82, 103, 124 and 130 units per ml.

$ZnSO_4 \cdot 7H_2O$, 0.00015 M. (0.044 gm./L.); $C_6H_5-CH_2COOH$, 0.00183 M. (0.25 gm./L.); KH_2PO_4 ; $MgSO_4 \cdot 7H_2O$; and $NaNO_3$. The combined concentration of the latter three salts was 0.04 M. in all solutions, but the proportions of the three were varied over wide ranges. This concentration was chosen because it conformed to that of the solution already in use in numerous laboratories. The solutions were adjusted to pH 5.2–5.3 by addition of 10 N. NaOH before autoclaving. They were autoclaved for 20 minutes at 15 pounds pressure and 118°C. The pH after autoclaving was 5.0–5.1.

Cultures were inoculated by a "dry spore" technique in which five-day old sporulating cultures of the mold on bran are broken up into a coarse powder which is then insufflated into the culture bottles by means of a flock gun under 30 pounds pressure of sterile compressed air. The chief advantage of this method is that nearly all of the spores float on the surface of the solutions and, germinating rapidly, give rise to early, luxuriant mycelial growth uniformly distributed over the surface of the culture. It is impossible with this method to control the size of the inoculum as precisely as when liquid spore

suspensions are used but, since a large excess of spores was inoculated into each culture, this factor was considered to be inconsequential.

Bottles were stacked in an incubator room thermostatically controlled at $23.5^\circ \pm 1^\circ C$. Triplicate cultures were inoculated and were sampled daily from the second through the seventh days by withdrawing approximately 0.5 ml. liquor from each culture. The samples were pooled and were assayed daily in quadruplicate by the standard cylinder plate method employing *Staph. aureus*, NRRL strain No. 313 as the test organism. Potencies are expressed in Oxford units.

Table 1 shows the molecular proportions of each of the three principal salts in each of the sixty-five solutions that were tested. Figure 1 shows graphically the molecular composition of the sixty-five solutions, each number within the triangle corresponding to a different culture. Reference to this figure facilitates locating the different cultures in the subsequent diagrams.

RESULTS OF EXPERIMENTS.—*Influence of relative concentrations of salts on penicillin production when total concentration of salts remains constant.*—The

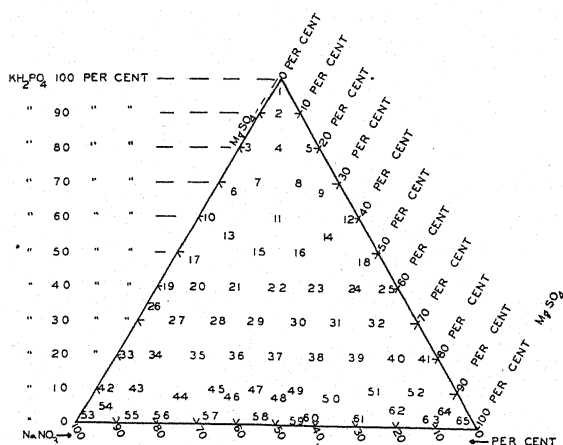


Fig. 1. Chart to show graphically the composition of the different solutions tested. The total molar concentration of the three principal salts in each solution = 0.04. Thus each apex of the triangle represents a concentration of 0.04 M. of the particular salt. Reference to this figure facilitates locating the different cultures in figures 2 to 5. Each number within the triangle corresponds to a different culture.

results of these experiments show that pronounced differences in the synthesis and/or accumulation of penicillin in surface cultures may be occasioned merely by changing the proportions of inorganic nutrients furnished in the culture solution.

The potency in the cultures on solution No. 42 on each day was equated to one, and the relative potency in each of the other cultures was calculated on this basis.⁴ The relative potencies from days four to seven are presented in table 2 and are shown diagrammatically in figures 2 to 5.

The numbers within the triangles in figures 2 to 5 represent the relative concentrations of penicillin in the different cultures, expressed as decimal fractions of that in the standard solution. The short dashes on the sides of the different triangles mark off ten per cent intervals. Each diagram has been divided arbitrarily into three zones, depending upon the concentration of penicillin accumulated in the several cultures at the time of assay. All solutions in which the relative penicillin concentration was between 0.9 and 1.1 are classed together. These are considered "average." Those solutions which were definitely inferior to the standard (relative concentration 0.89 or less) are grouped together and those that seemed definitely superior to the standard (relative concentration 1.11 or greater) have been placed in another group. These different areas are cross hatched in different ways to distinguish one from the other and to facilitate tracing the zones through the several diagrams.

The present data are not offered as an infallible picture of penicillin formation and/or accumulation

⁴ Solution No. 42 was chosen as the standard because this was the formula that was used routinely in many laboratories.

TABLE 3. Solutions that excel the standard solution by eleven per cent or more in production of penicillin for one or more days during the fermentation cycle.

Solution number	Day on which higher yield obtained	Molecular proportions		
		KH ₂ PO ₄	MgSO ₄	NaNO ₃
17	4th to 7th.....	47.5	5.0	47.5
20	4th to 7th.....	40.0	10.0	50.0
21	5th to 7th.....	40.0	20.0	40.0
31	4th to 6th.....	30.0	50.0	20.0
15	5th and 6th.....	50.0	20.0	30.0
29	5th and 6th.....	30.0	30.0	40.0
39	5th and 6th.....	20.0	60.0	20.0
11	6th and 7th.....	60.0	20.0	20.0
22	6th and 7th.....	40.0	30.0	30.0
28	6th and 7th.....	30.0	20.0	50.0
37	6th and 7th.....	20.0	40.0	40.0
7	4th only	70.0	10.0	20.0
27	4th only	30.0	10.0	60.0
38	4th only	20.0	77.5	2.5
45	4th only	10.0	30.0	60.0
57	4th only	2.5	32.5	5.0
5	5th only	80.0	17.5	2.5
10	5th only	60.0	2.5	37.5
13	5th only	55.0	10.0	35.0
40	5th only	20.0	70.0	10.0
41	5th only	20.0	77.5	2.5
35	6th only	20.0	20.0	60.0
44	6th only	7.5	22.5	70.0
4	7th only	80.0	10.0	10.0
9	7th only	67.5	27.5	5.0
30	4th and 6th.....	30.0	40.0	30.0
8	4th and 7th.....	70.0	20.0	10.0

under the conditions of the experiment, but they do serve to illustrate certain relations that exist between the inorganic composition of the medium and the accumulation of penicillin therein.

Although production of penicillin is of immediate practical importance, the chief permanent value of the influence noted here on penicillin accumulation may reside in the possible significance of these studies for fundamental research on metabolism of molds and perhaps of other organisms.

The diagrammatic presentation of the data is somewhat subjective, since the contour lines were located merely by inspection. More objective methods could have been used to determine the positions of the boundaries between the zones, but since experiments of this kind are subject to rather large experimental errors, it seemed that further refinement of the graphical representation was not warranted by the experimental data.

Although the true picture may differ slightly from the graphic representation, the diagrams are

useful for showing trends that are not so readily apparent from examination of the data in tabular form.

Attention should be focused on the clear zones, since these are the zones of distinctly superior solutions. The general contours and the areas of these zones changed from day to day, probably reflecting changing metabolic requirements and activity of the mold at different stages of development.

Considering the yields of penicillin in the cultures from the fourth through the seventh day of fermentation as a criterion, twenty-seven of the solutions that were tested excelled the standard by more than ten per cent on one or more of these days. In only three of these did KH_2PO_4 comprise less than twenty per cent of the total salt concentration; in only two of them was the concentration of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ less than ten per cent of the total salt concentration, and in none of them did NaNO_3 account for more than seventy per cent of the total

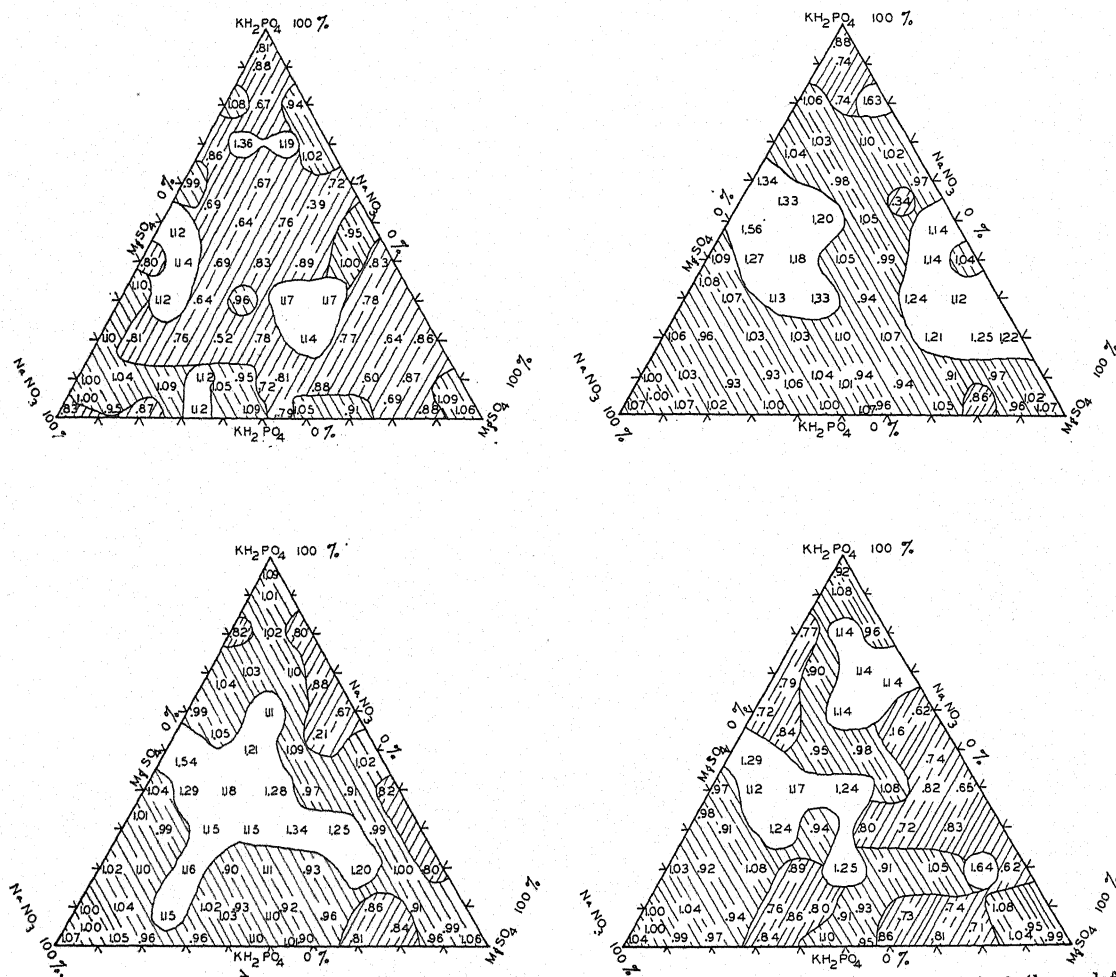


Fig. 2-5. Diagrammatic representation of the relative potency after 4 (upper left), 5 (upper right), 6 (lower left), and 7 days (lower right), respectively, in the solutions studied. The numbers within the triangles represent the potency in the different cultures, expressed as decimal fractions of that in the standard solution (No. 42) at the same time. The short dashes on the sides of the different triangles mark off ten per cent intervals.

salt concentration. These facts are set forth in table 3 and are shown graphically in figure 6 (lower part). These figures differ markedly from the formula that was recommended previously in which the relative concentrations of KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and NaNO_3 were 0.09, 0.025, and 0.885, respectively.

Twelve of the combinations of salts shown in table 3 yielded 100 or more units of penicillin per ml. of crude liquor on the fifth day of fermentation. The compositions of these solutions are summarized in table 4 and are treated graphically in figure 6 (upper part).

There are two prominent features of these solutions. In none of them did KH_2PO_4 comprise less than twenty per cent nor NaNO_3 comprise more than fifty per cent of the total salt concentration. Thus, these values may be set as approximate limits required for maximum synthesis and/or accumulation of penicillin in this kind of medium. The proportion of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in the solution, although important, appears to be less critical, since high-yielding solutions were found over a wider range of relative concentrations of this salt than of the other two (fig. 6).

A comparison is shown in figure 7 of the course of increase in potency in the former standard solution (No. 42) and in solution No. 17. It is clear that the superiority of the improved solution lies in its ability to accelerate production of penicillin during the first phase of fermentation. During the second phase the curves are nearly identical in slope, indicating that the factors operating to govern the increase in potency during this period are essentially the same in the two solutions.

The curves that are shown are typical for penicillin accumulation. There seem to be two distinct phases in the fermentation cycle. The curve for the first phase, lasting from the second to the fifth day, is characterized by a relatively steep slope. During the second phase which lasts from the fifth through the seventh day the potency increases much more

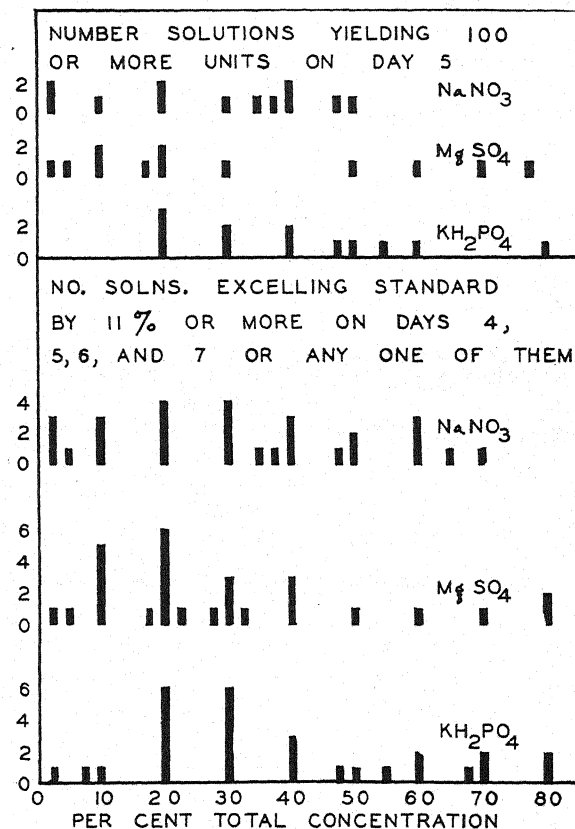


Fig. 6. (Upper portion) Histograms to show the number of solutions with each percentage of the different salts that were used that yielded potencies of 100 or more units on day 5. (Lower portion) Histograms to show the number of solutions with each percentage of the different salts that were used in which the potency excelled that in the standard solution (No. 42) by eleven per cent or more on days 4, 5, 6, and 7 or any one of them.

slowly. It is not known whether this is because synthesis by the mold is decreased during this period

TABLE 4. Solutions that yielded 100 units or more penicillin on the fifth day of fermentation.

Solution number	Units/ml. on day 5	Molecular proportions			Millimoles/L. ^a		
		KH_2PO_4	MgSO_4	NaNO_3	$\text{PO}_4^{=}$	$\text{Mg}^{++}\text{SO}_4^{=}$	NO_3^-
5	134	80.0	17.5	2.5	32	7	1
10	110	60.0	2.5	37.5	24	1	15
13	109	55.0	10.0	35.0	22	4	14
15	100	50.0	20.0	30.0	20	4.8	12
17	128	47.5	5.0	47.5	19	2	19
20	104	40.0	10.0	50.0	16	4	20
21	99	40.0	20.0	40.0	16	8	16
29	109	30.0	30.0	40.0	12	12	16
31	102	30.0	50.0	20.0	12	20	8
39	100	20.0	60.0	20.0	8	24	8
40	102	20.0	70.0	10.0	8	28	4
41	100	20.0	77.5	2.5	8	31	1

^a The corn steep liquor increased these values approximately as follows: $\text{PO}_4^{=}$, 1.1; $\text{SO}_4^{=}$, 17; Mg^{++} , 29; NO_3^- , 5.1.

TABLE 5. Accumulation of penicillin in solution No. 17 prepared in different total salt concentrations.

Total salt conc.	Units/ml. on day 7	Max. units/ml.	Day of max.	No. of days between 5th and 10th that potency exceeded 100 u/ml.	Fr. wt. (gms.) mold on day 11
0.0010 M.	90	111	9	2	57.0
0.0020 M.	100	112	8	2	58.2
0.0040 M.	112	116	8	3	55.7
0.0050 M.	113	136	8	2	55.7
0.0066 M.	108	126	8	2	57.6
0.0100 M.	112	133	8	3	59.0
0.0200 M.	117	117	7	3	55.7
0.0400 M.	123	123	7	3	53.0
0.0800 M.	136	136	7	6	63.0
0.1200 M.	140	140	7	5	61.3
0.1600 M.	132	146	8	4	60.0
0.2000 M.	124	146	8	4	52.0
0.3000 M.	110	125	8	4	...
0.4000 M.	52	91	9	0	...
0.6000 M.	12	49	13	0	...

or whether the retarded rate of increase is due to the onset about the fifth day of the destructive forces that cause deterioration of the penicillin already accumulated in the medium.

Influence of the total concentration of salts on penicillin production when relative concentrations of the salts remain constant.—All of the experiments described in the foregoing section were carried out at a total salt concentration of 0.04 M.⁵ This concentration was chosen because it corresponded to that of the solution that was currently in use when the experiments were begun.

It seemed of interest to determine whether the production of penicillin could be increased further by alteration of the total salt concentration. Accordingly, a series of experiments was set up in which only the total concentration of the salts was varied, the relative concentrations remaining constant. Since solution No. 17 furnished the highest yields in the earlier experiments, it was selected for this investigation. Phenylacetic acid was omitted from the medium, however. Thus, the maximum potencies attained were lower than in the earlier experiments. The results, presented in figure 8 and table 5, show that gains of ten to fifteen per cent in the yield on the seventh day were obtained when the total salt concentration was raised from 0.04 M. to 0.08 or 0.12 M.

The critical importance of $\text{PO}_4^{=}$ for penicillin production, which has become increasingly apparent throughout the course of these experiments and more especially in others concerned exclusively with synthetic media that will be reported later, is manifest in these data. Abundant evidence has been accumulated in the experiments on synthetic media that increasing the $\text{PO}_4^{=}$ level in the fermentation

⁵ This is neglecting the $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ which was present in all solutions in a concentration of 0.00015 M., and the salts contributed by the corn steep liquor.

medium, within certain limits, prolongs the time during which maximum potency obtains therein. The data in table 5 (column 5) are interesting in this connection. In the standard culture solution (0.04 M.) the potency generally builds up comparatively rapidly until the fifth day of fermentation and then increases slowly for the next two days (see fig. 7) after which it drops abruptly, usually to less than 100 units/ml. by the eighth day, when phenylacetic acid is omitted from the medium. Thus the assays usually read over 100 units/ml. for two

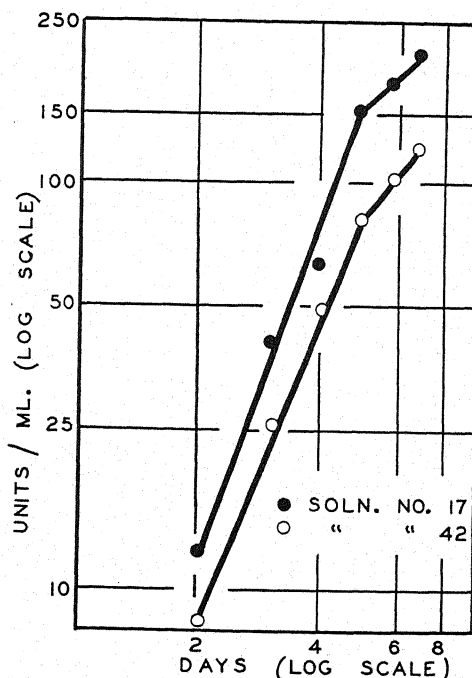


Fig. 7. Course of increase in potency in former standard solution (No. 42) and in best solution (No. 17).

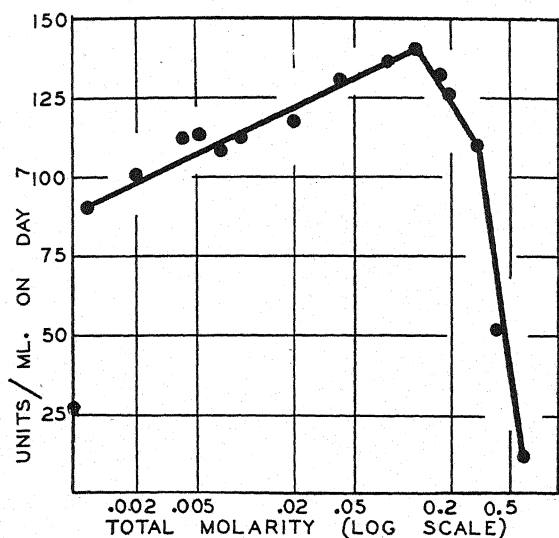


Fig. 8. Potency on day 7 as a function of total molar concentration (KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and NaNO_3) in solutions with the following proportional composition: KH_2PO_4 , 0.475; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05; NaNO_3 , 0.475.

or three days only. When the total concentration (and hence $\text{PO}_4^{=}$ concentration) was doubled, however, the maximum potency was maintained for three days and the assays read over 100 u/ml. from the fifth through the tenth days. Likewise, in all of the more concentrated solutions that were suitable for production of penicillin the potency remained relatively high for at least four days.

It is not clear at present whether this was due to greater stability of the penicillin that was produced or to a prolonged period of production which continued actively even after deterioration of the already formed penicillin had become appreciable. This question is now under investigation. This result was not brought about by greater buffering due to increased concentration of KH_2PO_4 and hence maintenance of a more favorable pH, since the pH values were essentially the same in all cultures during this period.

The complete series of experiments reported in this paper has been duplicated in submerged cultures with several strains of *Penicillium chrysogenum*. Variations in yield that were brought about by changes in the inorganic composition of the medium

were of the same order of magnitude as those reported above. The optimum relative concentrations of salts were different, however. Data from experiments with submerged cultures have not yet been passed for publication.

SUMMARY

A study was made of the accumulation of penicillin in cultures of *Penicillium notatum* grown in sixty-five different nutrient solutions containing KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and NaNO_3 in different proportions but all with a total molar concentration (of these three salts) of 0.04 M. The solutions contained in addition lactose, corn steep liquor, zinc sulphate and phenylacetic acid. The results emphasize the importance of obtaining a proper balance among the concentrations of $\text{PO}_4^{=}$, Mg^{++} , $\text{SO}_4^{=}$, and NO_3^- in the solutions if maximum yields of penicillin are desired. For a given level of $\text{PO}_4^{=}$, as the concentration of Mg^{++} and $\text{SO}_4^{=}$ was raised, it was necessary to lower the concentration of NO_3^- . The best solutions, from the standpoint of penicillin production, contained not less than 8 millimoles KH_2PO_4 per liter and not more than 20 millimoles NaNO_3 per liter. The absolute concentrations in the optimum solution in this series were KH_2PO_4 , 0.019 M.; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 M.; and NaNO_3 , 0.019 M. The proportions of the three salts in the optimum solution were KH_2PO_4 , 0.475; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05; and NaNO_3 , 0.475.

In a series of solutions with these relative concentrations for the three salts, maximum potency on the seventh day was obtained at a total concentration of 0.12 M.

CUTTER LABORATORIES,
BERKELEY, CALIFORNIA

LITERATURE CITED

- ABRAHAM, E. P., AND E. CHAIN. 1942. Purification and some physical and chemical properties of penicillin. *British Jour. Exper. Path.* 23: 103-115.
- , ET AL. 1941. Further observations on penicillin. *Lancet* 141: 177-188.
- CHALLINOR, S. W., AND J. MACNAUGHTON. 1943. The production of penicillin. *Jour. Path. and Bact.* 55: 441-446.
- DIMOND, A. E., AND G. L. PELTIER. 1945. Controlling the pH of cultures of *Penicillium notatum* through its carbon and nitrogen nutrition. *Amer. Jour. Bot.* 32: 46-50.

HABIT OF GROWTH OF RUBUS ROSAEFOLIUS SMITH IN HAWAII¹

Charles J. Engard

RUBUS ROSAEFOLIUS Smith is a plant of wide distribution in the tropics and has become established in more temperate climates. Rydberg (1913) states that the species is "native of southern and eastern Asia," and that the type locality is the "Island of Mauritius," although it was introduced there from the Malay Isles.

There is contradiction in the literature as regards the duration of life of the canes. Focke (1911, p. 149) places the species under "Caules annui" in his key to the section *Rosaeifolii*. Rydberg (1913, p. 441) begins his description of the species with "stems perennial, erect. . . ." Bailey (1923, p. 149) describes the plant as "a vigorous evergreen species . . .," and again (1925, p. 3028) as "erect and tall-growing, evergreen in warm countries. . . ." No reference is made to the duration of the canes. The keys included in these publications do not use the annual or perennial nature of the canes in the identification of the species. In his manual, however, Bailey (1924) separates *R. rosaeifolius* from *R. illecebrosus* on the basis of the biennial or perennial canes of the former and the annual canes of the latter species.

Dr. H. St. John of the Botany Department, University of Hawaii, called my attention to this taxonomic discrepancy when I inquired into the growth habit of *R. rosaeifolius*. The following is a report of the investigation that resulted.

INVESTIGATION.—*Rubus rosaeifolius*, known locally as thimbleberry, is now abundant in Hawaii, especially at altitudes above 300 meters. It spreads rapidly by seeds and by horizontal roots. In outward appearance the colony is evergreen, but closer inspection reveals a mat of prostrate, dead and dying canes, effectively hidden from casual sight by the ascending² vegetative and fruiting canes. Flowers and fruits are produced throughout the year, but more abundantly during the spring and summer months. Flowers, and later the fruits, are produced on a stem in basipetal succession (table 1).

On October 10, 1943, eight vigorous canes were labeled with paraffined, metal-rimmed tags on Mt. Tantalus, Oahu. These plants were not checked again until March 9, 1944. Growth had been unexpectedly rapid (although it appears that the plants grow least rapidly in the winter months), and a few of the labels could not be found. Some labels had rotted owing to the fact that heavy rainfall is characteristic of the locality. Since it appeared that the life of canes was of short duration, a closer series of observations was instituted. Those tagged plants that could be found, and some new ones, making a total of fourteen, were labelled by tying strong cords, identified by knots, on the chosen,

ascending canes. The ends of the cords were brought together at a convenient spot and the plants were easily found at subsequent periods of observation.

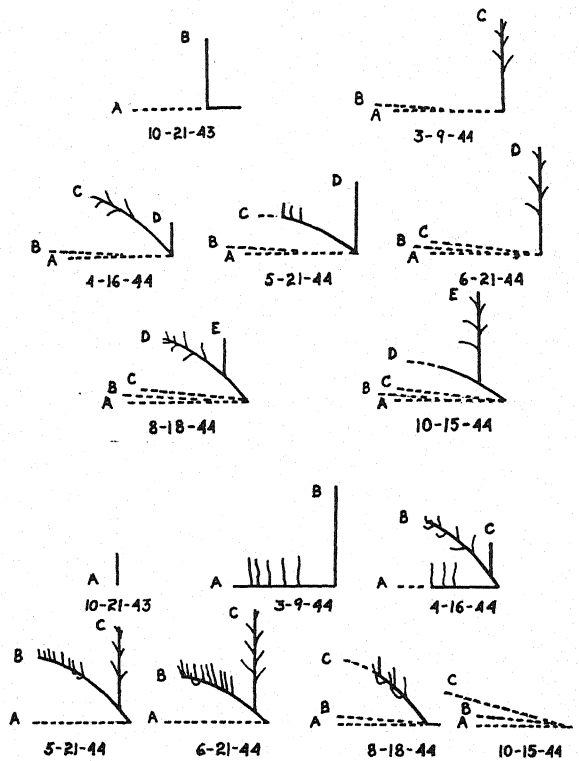


Fig. 1 and 2. Diagrammatic series depicting growth of canes of *Rubus rosaeifolius* Smith during one year. Dotted lines represent dead canes or portions of canes; solid lines represent living canes. Canes with short branches represent fruiting or flowering. Succession of cane growth is indicated by letters. The date is indicated under each diagram.—Fig. 1 (above). Cane No. 1 in table 1.—Fig. 2 (below). Cane No. 4.

Data for two of these are recorded in table 1. Their growth habits are shown schematically in figures 1 and 2. It is apparent that four or five canes become the ascending fruiting portion of the plant during a given year. Each cane has a duration of life of from one to two months. Death of the cane is basipetal. Frequently an axillary bud low on the fruiting cane will produce a vegetative branch. This becomes ascending while the parent cane becomes arched (fig. 1 and 3). It produces flowers and fruits at about the time that the parent cane becomes prostrate. Another vegetative cane may develop on the second, now fruiting vertical cane, and the previously described history is repeated; or, frequently, a new vegetative shoot develops from the base of the original now prostrate, cane. This point of origin is the crown, the thickened persistent base of a stem.

¹ Received for publication April 12, 1945.

² The terms ascending, arched, and prostrate are used in accordance with those of Bailey (1932).

TABLE 1. *Growth data on two plants.*

	Plant No. 1 (fig. 1)	Plant No. 4 (fig. 2)
Oct. 21, 1943	A, prostrate cane 90 cm. in length, dead to 30 cm. from basal end, where vegetative shoot B takes origin.	Cane A is vegetative, 30 cm. in height.
Mar. 9, 1944	A, cane nearly buried, rotting; B now prostrate, dead. At base of cane A is vegetative cane C, 100 cm. in height.	A is leafless, prostrate, with short, fruit-bearing branches. Terminal fruits dropped. At base of A is B, a vegetative, ascending cane 90 cm. in height.
Apr. 16	C is arched, bears fruit on many short branches. Young, vegetative cane D originates from base of C.	Cane A is dead 30 cm. from tip; has 3 short chlorotic branches. B is arched, has short flowering branches. At base of B is vegetative cane C, 30 cm. in height.
May 21	C is nearly prostrate, with 15 cm. of tip dead. Cane D, 60 cm. in height, is vegetative.	Cane B reclining, C is 70 cm. in height, in fruit (at top), and flower. Cane A dead to 15 cm. from basal end.
June 21	D is 90 cm. in height. Canes A, B, C are prostrate, dead.	Cane B has many short, fruiting branches, terminal fruits oldest, progressively younger fruits toward base. Cane C, 90 cm. in height, bears fruit.
Aug. 18	D arched, bears fruit on short, upper branches, flowers on lower. Lowermost branch, E, 30 cm. above base of D, is ascending, ^a vegetative, 30 cm. in height.	B is prostrate, dead to 15 cm. from base. Cane A is rotting. C is arched, has 15 cm. of tip dead.
Oct. 15	D is arched, bears fruit on lower branches, and has 30 cm. of tip dead. Cane E is 90 cm. in height, bears fruit.	All canes dead. No visible new canes from base.

^a Ascending is represented by vertical lines in diagrams.

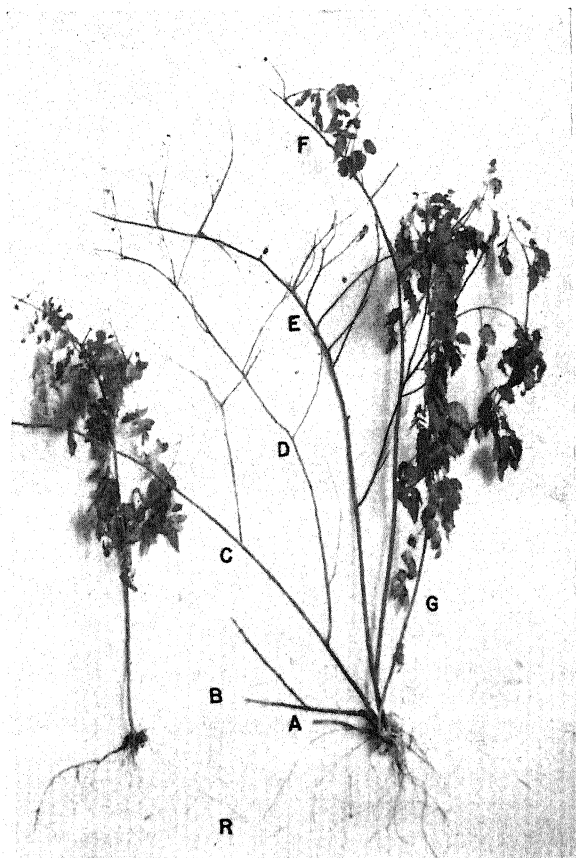


Figure 3 is a photograph of a plant which shows the growth habit of this species. The labelling and the legend indicate the sequence of development of the canes.

DISCUSSION.—Because terminology of the vegetative structure in *Rubus* was confused and inexact, Bailey (1932, p. 279) introduced the terms *primocane* for the cane in its first year, and *floricane* for the same cane in its second year. Later Bailey (1941) added the terms *parcifrond* to usually sterile, long leafy shoots on a floricane below the ordinary floral branches and *novirame* to a "flowering or fruiting axis or shoot on a primocane." The terms *caudex* and *crown* have been used in reference to the Rubi, the former term by Focke (1911) and the latter by Bailey (1941, p. 11). Caudex is defined by Jackson (1928) as "the axis of a plant, consisting of stem and root" and by Gray (1887) as "an upright or short rootstock." Crown is defined by Jackson (1928) as "the point where root and shoot meet."

The data and figures indicate (1) that a portion of a cane remains alive for a major part of the year in some plants, and the ascending fruiting canes are elongate branches derived from axillary buds low on the arched or prostrate foregoing canes as in figure 1, canes B, E; figure 2, C; figure 3, D; (2)

Fig. 3. Photograph of a plant of *R. rosaefolius* Smith, showing succession of canes (A to G). Cane G is vegetative. All others have fruited and canes A to E are dead. Cane A, the first one, has rotted away through almost its entire length. Horizontal root R leads to new stem about 70 cm. from parent plant.

other ascending fruiting canes are derived from a common base at ground level, such as canes C and D in figure 1, B in figure 2, and A, B, C, E, F, and G in figure 3;³ (3) some canes are vegetative for a period, then produce fruit, and finally give rise to low axillary shoots which are in turn vegetative, and fruiting.

The terms caudex and rootstock are too broadly defined to apply to this species. Crown, however, is useful, for it expresses precisely what is referred to in describing cane development in *Rubus*. The terms primocane, and floricanes defined in respect to the annual and biennial canes of the Rubi of the temperate zone, are not applicable to *R. rosaefolius* in Hawaii. To attempt to modify these terms which refer precisely to growth of canes with two distinct morphological stages separated by a dormant period, would be to lessen their value. Because growth of *R. rosaefolius* is characterized by the frequent production of new canes at, or near, the base of old prostrate ones, a new term is suggested. The cane thus produced is a *paucirame* (*paucus*-few, *ramus*-branch)—a branch of short duration, passing through vegetative, flowering, and fruiting stages in a space of a few months. It then dies back from the tip, but the base later produces a lateral ascending branch which continues the growth of the plant.

The plant is perennial, and propagates vegeta-

³ The plant in figure 3 was unusually prominent in respect to this characteristic; the method of growth mentioned in (1) is more dominant.

tively by long horizontal roots such as the one shown at R, figure 3. Microscopic examination of a section taken from a point opposite the letter A on the photograph proved that it was a root and not a rhizome.⁴ The canes are of only a few months' duration.

SUMMARY

The habit of growth of 14 plants of *Rubus rosaefolius* Smith in Hawaii was studied. The plant is perennial, and produces four or five canes during a given year. These canes take origin from a node near the basal end of a previously fruiting cane or from the crown at ground level. Each cane passes through, first, a vegetative stage and then a fruiting stage, the sequence being of only a few months' duration. Flowering, fruiting, and death of each cane are basipetal.

Terminology as applied to the Rubi is discussed. The terms primocane and floricanes, originally applied to the biennial temperate zone Rubi, are found unsuitable for use in reference to *R. rosaefolius* in Hawaii. The term *paucirame*, a branch of few months' duration, is suggested for this species.

The colony of *R. rosaefolius* increases in size by means of horizontal roots from which new shoots arise.

DEPARTMENT OF BOTANY,
UNIVERSITY OF HAWAII,
HONOLULU, T. H.

⁴ I have come across no mention of propagation by this method in the literature. Expansion of a colony by stolons has been mentioned (Bailey, 1925, p. 3023).

LITERATURE CITED

- BAILEY, L. H. 1923. *Quidam Rubi cultorum*. Gentes Herbarum I.
———. 1924. *Manual of cultivated plants*. Macmillan, New York.
———. 1925. *The standard cyclopedia of horticulture*. Macmillan, New York.
———. 1932. *Eubati Boreali-Americani*. Gentes Herbarum II.
———. 1941. *Species Batorum I*. Gentes Herbarum V.
———. 1944. *Certain Rubi of the occidental tropics*. Gentes Herbarum VI.
FOCKE, W. O. 1911. *Species Ruborum II*. Stuttgart.
GRAY, ASA. 1887. *Gray's lessons in botany*. American Book Company, New York.
JACKSON, B. D. 1928. *A glossary of botanic terms*. Duckworth, London.
RYDBERG, P. A. 1913. *North American Flora* 22. Rosaceae. New York Botanical Garden.

SILICON ABSORPTION BY RYE AND SUNFLOWER ¹

Robert T. Whittenberger

THE PRESENCE of large quantities of silicon in many plant species has stimulated considerable research on the essentiality and function of this element (see Wagner, 1940, for literature review). Yet the equally interesting question as to the manner of its absorption and accumulation has been largely ignored. Although plants normally grow in a silicon-rich substrate, the way in which they absorb this element in quantity is not readily apparent. At ordinary soil temperatures and hydrogen-ion concentrations, most of the naturally occurring compounds of silicon commonly are regarded as either insoluble or colloidal, and should therefore be unavailable for absorption.

In the present study certain factors thought capable of influencing silicon absorption have been investigated. The factor of soluble silicon concentration was given special attention, since it is possible that plants accumulate large amounts of silicon from very dilute solutions, as has been demonstrated for selenium by Byers (1935) and Trelease and Trelease (1938). On the other hand, in accordance with modern concepts of solute absorption (Steward,

er, 1930; Wherry, 1932). Is it possible that colloidal forms of silicon can pass the cell membranes?

Species absorb widely different amounts of silicon from the same soil (Richardson, 1920; Wherry, 1932), indicating that they differ inherently either in their capacities to absorb silicon or in their capacities to alter the soil siliceous materials, or both. In the present paper attention is given these concepts by studying not only the effect of different siliceous materials upon silicon absorption by the plants, but also the effect of the plants upon silicon in the materials. An opportunity was afforded also for inquiring into the effect of hydrogen-ion concentration on growth at different seasons, and into the essential nature of silicon as a nutrient.

MATERIALS AND METHODS.—Fruits of Rosen rye (*Secale cereale* L.) and Mammoth Russian sunflower (*Helianthus annuus* L.) were germinated on paraffined screens over pyrex dishes containing half-strength nutrient solution. After six days, selected seedlings were transferred to the cultures.

The following basal nutrient solutions were utilized:

A		B		C	
Salt	Molarity	Salt	Molarity	Salt	Molarity
KNO ₃	.0078	KH ₂ PO ₄	.004	K ₂ HPO ₄	.004
(NH ₄) ₂ SO ₄	.0032	NH ₄ NO ₃	.004	NH ₄ NO ₃	.003
Ca(H ₂ PO ₄) ₂ ·H ₂ O	.0006	Ca(NO ₃) ₂ ·H ₂ O	.001	Ca(NO ₃) ₂ ·H ₂ O	.001
MgSO ₄ ·7H ₂ O	.0005	MgSO ₄ ·7H ₂ O	.001	MgSO ₄ ·7H ₂ O	.001

1935; Hoagland, 1937; Hoagland and Arnon, 1941), a substance, although soluble, may not be available to plants.

Because of the important effect of hydrogen-ion concentration on both solute absorption (Lundegardh, 1934; Osterhout, 1936; Hoagland, 1937) and solubility of soil siliceous compounds, this factor was studied from two viewpoints. Most siliceous compounds are dissolved slowly by strong alkalis. In acid media, however, they are either highly insoluble or else decompose slowly, yielding silicic acids which ordinarily are considered incapable of passing parchment paper or collodion membranes. Yet it is known that plants do take up appreciable quantities of silicon from distinctly acid soils (Coop-

To each solution were added boron, manganese, zinc, copper, and molybdenum to give final concentrations of 0.5, 0.5, 0.05, 0.02, and 0.01 p.p.m., respectively. Solution A contained some colloidal material; iron, as ferric tartrate, was maintained at 5 p.p.m. in this nutrient. Solutions B and C contained no iron and were free from precipitate and colloidal matter except for a slight opalescence at the highest pH and highest silicon concentration employed. Iron was applied effectively as a spray of acidified 0.1 per cent ferrous sulfate, at five- or six-day intervals, to the shoots of plants growing in these solutions. Solutions were made relatively low in calcium and magnesium, owing to the tendency of these elements to form insoluble salts with the silicate ion (MacIntire, Shaw, and Young, 1925; Kardos and Joffe, 1938). In series A, AA (tables 1 and 2) and B, BB (table 3) silicon was added to the basal solution in the form of synthetic, crystalloidal sodium metasilicate, Na₂SiO₃·5H₂O, obtained from the Philadelphia Quartz Company.

Plants of the liquid culture series (tables 1–3, excluding series A6, AA6, B5, and BB5) were grown

¹ Received for publication May 3, 1945.

This dissertation was submitted to the Department of Botany, University of Pennsylvania, Philadelphia, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Acknowledgment is made to Dr. Edgar T. Wherry, Dr. J. R. Schramm, Dr. William Seifriz, Dr. Paul Allen, and the other members of the Department of Botany for their assistance and criticism during the study or in the preparation of the manuscript.

in two-quart jars. Nutrient solutions were changed weekly, and the pH was kept relatively constant (± 0.2 of a unit) by daily adjustment with sodium hydroxide or hydrochloric acid. In series A6 and AA6 (table 1) a silicon-free solid substrate was achieved by the use of granular, 30-mesh, polymerized methylmethacrylate, a plastic called Crystalite. This white compound had a specific gravity of about 1.2 and showed a field water-holding capacity of 53 per cent as compared with 16 per cent for the quartz sand used. Preliminary experiments with Crystalite had shown it to be free from silicon and non-toxic. Plants of the Crystalite series, grown in pots coated with acid- and alkali-resistant paint, were treated daily with the appropriate nutrient.

In series B5 and BB5 (table 3) plants were grown in painted pots containing 25-mesh acid-washed quartz sand. Fitted into the basal hole of each pot was a siphon with a pinch-clamp, permitting collection of the "soil" solution for silicon analysis. A total of 300 ml. of nutrient B containing no silicon was flushed through each pot daily at three intervals, and the sand, except at the time of draining, was kept saturated with nutrient. In series C2, CC2, and C3 (table 6) plants were grown with their

roots in 350 ml. collodion sacs suspended in beakers. Each sac was fastened at its open end to a large cork which held the plants in place. In the first arrangement of series C2, CC2, and C3, sacs were filled with a suspension of kaolin or bentonite in nutrient B and then were hung in a beaker containing 650 ml. of distilled water. Controls containing no plants were arranged similarly. In the second arrangement the positions of the distilled water and clay suspensions were reversed, so that the roots were immersed in distilled water and were separated from the clays by a collodion membrane. All clay suspensions were agitated and aerated daily by alternate lifting and ejection from a pipette, and by addition of nutrient solution to replace that lost by absorption and evaporation. At the termination of the experiment, tests showed all membranes to be identical with regard to silicon permeability. All plants were grown in an unshaded greenhouse whose temperature ranged from 60° to 95° F. in the spring and summer and from 55° to 70° F. in the fall and winter.

At the conclusion of each experiment, roots were washed repeatedly with distilled water and blotted for five minutes before determination of fresh weights. It was estimated that in the sand and Crys-

TABLE 1. *Effect of concentration on absorption and distribution of silicon in rye and sunflower.*

Series ^a	Season grown	Days grown	No. of plants	Shoots		Roots	Entire plant		
				P.p.m. Si in nutr. soln.	P.p.m. Si in fresh wt.	P.p.m. Si in fresh wt.	Dry wt. gms.	Ash gms.	P.p.m. Si in fresh wt.
A2	spring	30	12	0	048	.093
				50	40275	.178
				150	2059	1.14	.245
				450	1055	1.39	.296
AA2	spring	30	8	0	33	61	5.01	.999	37
				50	640	294	6.71	1.313	570
				150	999	401	7.83	1.545	920
				450	1032	3129	6.56	1.458	1415
A3	winter	37	12	0	145	56	2.00	.410	112
				50	981	691	2.62	.518	873
				150	3316	939	3.06	.665	2475
				450	2531	4623	2.75	.566	3269
AA3	winter	37	6	0	75	0	2.19	.622	70
				50	537	579	3.30	.787	546
				150	761	509	3.29	.933	719
				450	696	2265	3.58	1.221	1069
A4	fall	46	18	150	3470	640	6.81	1.022	2704
				450	3194	2489	5.95	.809	3021
AA4	fall	46	8	150	981	887	4.97	.868	967
				450	1079	2335	5.69	.848	1289
A6	spring	50	9	0	47	0	4.68	.701	28
				50	1270	743	4.26	.582	1074
				150	2321	1485	3.31	.462	1985
				450	2013	2382	3.83	.456	2144
AA6	spring	50	3	0	9	0	3.10	.477	8
				50	1041	304	3.50	.511	901
				150	1284	1168	4.06	.562	1256
				450	962	3115	2.25	.229	1308

^a Single lettered series are rye, double lettered, sunflower. Series A2 and AA2 were grown in nutrient B at pH 4.6; series A3 and AA3 in nutrient C at pH 4.6; series A4 and AA4 in nutrient B at pH 4.6; and series A6 and AA6 in Crystalite with nutrient B at pH 4.6. Plants of each silicon level within a series were pooled before analysis.

talite cultures about 5 per cent of the rye and about 15 per cent of the sunflower roots, dry weight basis, were lost. Total silica was determined by a method described by Loomis and Shull (1937), dissolved silica by the method of Schwartz (1934), and soluble silica within the plants by the method of Viehoever and Prusky (1938).

For the treatment of soil materials with nitrogen and carbon dioxide gases (table 7), samples were mixed in flasks with an aqueous phase, and the flasks were connected in series so that the contents of each could be aerated and agitated at the same rate from a single gas source. Two identical series were treated simultaneously, one with nitrogen gas, the second with carbon dioxide. Samples taken for analysis from the supernatant layer of each flask were replaced by more of the original aqueous phase.

OBSERVATIONS.—Effect of silicon concentration on its absorption and distribution.—Soluble silicon was absorbed readily by both rye and sunflower at a rate which was in large measure a function of its concentration, maximum absorption occurring at the highest, or 450 p.p.m., level. This is shown in the last column of table 1. Differences in age, season, nutrient solution and substrate did not appreciably alter this result. Increases in silicon concentration at low levels were reflected in relatively large increases in silicon absorption, whereas at high levels increases resulted in only slightly augmented absorption. Probably the concentration promoting maximum absorption lies at a level even higher than 450 p.p.m.

The increased silicon absorption at the higher

silicon concentrations was accompanied in general by decreased absorption of other ash constituents. Thus, in series A3, cultures with 0, 50, 150 and 450 p.p.m. of silicon contained respectively 20, 19, 19, and 18 per cent of ash (dry weight basis), excluding silicon. Similar results were obtained on other plants by Wagner (1940). Conversely, Hampton and Albrecht (1943) found that the liberal absorption of potassium and calcium by soybean roots had an excluding effect upon silicon. Germar (1934), however, reported that a deficiency of potassium limited the intake of silicon in other species, whereas an excess of nitrogen favored its intake.

Neither species showed evidence of silicon deficiency (Raleigh, 1939; Wagner, 1940). Of the two species, rye invariably absorbed the greater amount of silicon. The increase in yield (in most cases) of the silicon-supplied plants over that of the controls was probably insignificant, and the data can not be used in support of the essentiality of this element. Similar results in yield were obtained when sodium chloride was substituted for sodium silicate in the nutrient. The small amount of silicon present in or on the control plants apparently came largely from atmospheric dust, since the corresponding roots contained even less or no silicon.

It is interesting to note that no evidence of toxicity was obtained even when silicon was furnished the plants in concentration as high as 450 p.p.m. In contrast, King and Davidson (1933) found that a concentration of 93 p.p.m. of silicon was toxic to diatoms. Regarding humans, Denny, Robson, and Irwin (1939) suggest that the disease silicosis, prev-

TABLE 2. *Effect of silicon concentration on its absorption and on the distribution of soluble and insoluble forms in rye and sunflower.*

Series ^a	P.p.m. Si in nutr. soln.	Total transpiration 3 weeks in ml.	Plant part	pH of expressed sap	Silicon		
					In expressed sap (soluble)		In fiber residue (insoluble)
					Per cent of total Si	P.p.m. total fresh wt.	P.p.m. total fresh wt.
A5	150	2003	Leaf	5.90	10	299	2592
			Culm ^b	5.96	18	612	2788
			Root	6.35	33	995	2031
			Entire plant	...	20	612	2438
A5	450	1829	Leaf	6.12	13	266	1784
			Culm ^b	6.08	19	383	1677
			Root	6.35	46	2751	3218
			Entire plant	...	35	1191	2274
AA5	150	974	Leaf	6.70	6	210	3106
			Stem	6.66	49	238	248
			Root	6.31	33	701	1396
			Entire plant	...	21	327	1172
AA5	450	876	Leaf	6.20	10	238	2167
			Stem	6.31	33	135	266
			Root	6.28	7	574	8313
			Entire plant	...	9	290	3106

^a Plants (series A5, rye; series AA5, sunflower) were grown in nutrient B during winter at pH 4.6 for 42 days, and, in addition to the normal daylight, were illuminated 8 hours daily with fluorescent lamps. Plants from each culture (18 rye or 8 sunflower) within a series were pooled before analysis.

^b The term "culm" is used to designate all plant tissue from the first node to a height of 10 cm.

alent among miners, is associated with some inherent property of silica rather than with its mechanical abrasive qualities.

The distribution of silicon within rye plants was influenced in an unexpected manner by variations in concentration of soluble silicon in the nutrient. Maximum accumulation in rye shoots occurred when the silicon level was 150 p.p.m. rather than 450 p.p.m. (table 1). However, the roots (and the plant as a whole) accumulated the most silicon at the 450 p.p.m. level. In series A5 (table 2), for example, increasing the silicon level from 150 p.p.m. to 450 p.p.m. decreased the fraction of total plant silicon in the shoots from 65 to 38 per cent and increased the fraction in the roots from 35 to 62 per cent. Apparently translocation to the shoots of the absorbed silicon was impaired in the latter culture. Similar differences in silicon distribution at high

silicon levels were not so apparent in sunflower shoots, although sunflower roots accumulated silicon in great excess from nutrient containing 450 p.p.m. of silicon.

No satisfactory explanation was found which would account for the differences in silicon distribution between the rye cultures containing 150 p.p.m. and 450 p.p.m. of silicon. The work of Schmidt (1936), Freeland (1936, 1937), and Wright (1939) indicating that the rate of transpiration affects absorption and distribution of certain mineral salts and that of Germar (1934) indicating that silicon deposition in leaves varies directly as the transpiration, were suggestive. Yet the difference in transpiration between the two cultures in the present case was slight and probably insignificant in this connection (table 2).

About 33 per cent of the silicon in the sunflower

TABLE 3. *Effect of hydrogen-ion concentration on silicon absorption and on growth of rye and sunflower.*

Series ^a	Season grown	Days grown	Number of plants	P.p.m. Si in nutr. soln.	pH	Entire plant	
						Dry wt. gms.	P.p.m. Si in fresh wt.
B1	winter	19	8	59	7.1	.24	1331
					5.5	.25	981
					4.6	.29	780
BB1	winter	19	6	59	7.1	.73	355
					5.5	1.05	257
					4.6	1.05	224
B2	winter	42	18	150	7.1	.77	4899
					5.3	3.88	2625
					4.6	4.67	2204
BB2	winter	42	8	150	7.1	4.92	1135
					5.3	5.75	733
					4.6	6.99	743
BB3	fall	16	6	150	6.7	.70	383
					5.3	.81	439
					4.6	.70
B4	winter-spring	42	18	150	7.1	.31 ^b	12492
					4.6	6.36	2172
					4.0	6.85	2424
BB4	winter-spring	42	8	150	3.6	3.10	2905
					7.1	17.77	1891
					4.6	13.35	1009
B5	winter-spring	58	18	...	4.0	13.54	1055
					3.6	8.03	1317
					7.1	24.96	346
BB5	winter-spring	58	8	...	5.3	24.10	262
					4.6	19.33	149
					3.8	15.75	163
B6	spring	42	18	0	7.1	33.72	154
					5.3	25.39	131
					4.6	24.57	135
BB6	spring	42	8	0	3.8	14.43	135
					7.1	17.11
					4.6	16.59
					7.1	27.67
					4.6	14.23

^a Single lettered series are rye, double lettered, sunflower. Series B1 and BB1 were grown in nutrient A; all other series were supplied with nutrient B. Series B5 and BB5 were grown in quartz sand; all other series were grown in liquid media. Plants of each treatment within a series were pooled before analysis.

^b Rye plants of pH 7.1 for the most part were dead at the end of 3 weeks.



Fig. 1 and 2.—Fig. 1. Sunflower (series BB4, table 3) showing late winter response to two hydrogen-ion concentrations. Age of plants, 3 weeks.—Fig. 2. Same plants as in figure 1, showing growth during early spring. Age of plants, 6 weeks.

root was soluble when the silicon level was 150 p.p.m., whereas only 7 per cent was soluble at the 450 p.p.m. level. Although the latter condition seemingly constituted an obstacle to silicon mobility, no such obstacle existed in the case of rye. That the mobility of silicon within the plants was probably not much affected by hydrogen-ion gradients was suggested by the similarity of the plant organs with respect to cell sap reaction (table 2). There was little difference in sap reaction either between plants receiving the two silicon concentrations or between organs of the same plant.

Of the total silicon absorbed by the plants of series A5 and AA5, the major portion was deposited in the insoluble state. With the exception of the sunflower culture containing 450 p.p.m. of silicon, a greater proportion of the total silicon in the leaves was insoluble than was the case with the other plant organs. Although sunflower stems contained the least fraction of total plant silicon of all organs, a large fraction of that present was soluble. Sunflower leaves contained an even greater concentration of silicon than did rye leaves. From the data on concentration of soluble silicon in roots, it is apparent that silicon was absorbed against a concentration gradient in both species.

Effect of hydrogen-ion concentration on silicon absorption and distribution.—Maximum absorption

of soluble silicon, expressed as p.p.m. of fresh weight, was favored in both species by a low concentration of hydrogen ions (table 3), although appreciable quantities also were absorbed at a relatively high concentration (pH 3.6). Repeated experiments under a variety of cultural and environmental conditions gave approximately the same comparable results. The only exception was in the short-termed series BB3 where sunflower absorbed slightly more silicon at pH 5.3 than at pH 6.7. Maximum absorption was largely independent of plant vigor, and occurred at a low hydrogen-ion concentration irrespective of whether the plants grew best or poorest at that level. In both species, a high concentration of hydrogen ions favored accumulation of silicon in stems rather than in leaves. An inhibitory effect of hydrogen ions on the translocation of silicon was thus suggested. For example, in series BB4 sunflower stems contained 9, 7, 6, and 5 per cent of the total shoot silicon (fresh weight basis) at pH treatments of 3.6, 4.0, 4.6, and 7.1, respectively.

Yields as affected by hydrogen-ion concentration at different seasons.—Arrhenius (1929) stated that the optimum pH for growth of rye was about 5.0. Recently, however, hydrogen-ion concentration has been regarded as a variable factor which may be modified extensively by cultural and climatic conditions. Arnon (1937, 1939) showed that growth of

barley was quantitatively and comparatively different at a given pH value in two seasons; plants that made best growth at pH 5 in the fall showed no advantage at that level of reaction in the spring.

Of the pH values used during the winter season, both rye and sunflower grew best at pH 4.6 and poorest at pH 7.1 (series B1 and BB1, table 3), irrespective of the presence or absence of silicon. The same comparisons were obtained the following winter, although the results in cultural conditions (series B2 and BB2) these series sunflower appeared less responsive in its pH requirements, and did not show repression in growth at pH 7.1.

The pH requirements of the plants during the growing season (spring and summer) were in contrast with those of winter. During spring, maximum yield of rye was obtained at pH 7.1, a pH inferior yield (series BB4, B5, BB5, table 3). Sunflower plants grown in spring showed a response intermediate between that of winter and spring, and showed no definite pH preference (series BB3). In series B4 and BB4, cul- tivation during late winter and continued through spring showing the transition between winter and spring responses (fig. 2). Owing to the death during late winter of the rye plants at pH 7.1, the spring response is missing. Rye plants gave comparatively better growth at low pH values (4.0 and 3.6) than did sunflower.

Silica absorption from various materials.—Wagner (1934) noted that silicon was taken up by barley in increasing amounts from quartz sand, loam soil, low moor soil, "sand," marl, and loess. Marshall (1934) likewise noted that only a small amount of silicon were absorbed by rye, barley, and sunflower grown in quartz sand. Raleigh (1939) found that the silicon in quartz sand was not readily available to the plants because he germinated in sand the best

seed to be used in silicon-deficiency studies. On the other hand, Viehoever and Prusky (1938) stated that the largest amount of silicon apparently is present in fully developed plants grown on sandy soil, and Richardson (1920) found high percentages of silicon in several species growing in sand dunes. Hoagland and Martin (1923) reported that appreciable amounts of silicon from sand entered the soil solution and plants. Wherry (1932) observed no regularity in the silicon content of certain species grown on sandy coastal-plain woods soil and on low-silicon serpentine barren soil; thus *Phlox subulata* contained more silicon when grown on the latter than on the former, while the converse was true with *Smilax rotundifolia*.

Only small quantities of silica (1 p.p.m. of silica = 0.467 p.p.m. of silicon) were taken up by either rye or sunflower from quartz sand (table 4). Much greater quantities were absorbed from sandy loam, kaolin, serpentine soil, and Crystalite supplied with soluble silica. In general, the amount of silica absorbed was correlated with its solubility, which was greatest in the Crystalite culture and least in the quartz sand. The solubility of silica in sand was indeed so low (0.7 p.p.m.) that it seemed difficult to account for even the small quantities of silica absorbed on the basis of solubility alone. Accordingly, the possibility of a solvent effect of the roots on silica, or the absorption of colloidal silica, was considered.

It was found in series B5 and BB5 (tables 3 and 5) that the roots exerted a solvent action, although slight, on quartz sand. More soluble silica was leached from sand supporting plants than from sand not supporting plants, irrespective of the reaction of the leachate (table 5). Greater quantities of silica were dissolved from the sand, with and without plants, at pH 7.1 than at pH 3.8. Slightly greater amounts were dissolved under rye, the species of

TABLE 4. Silica absorption from various materials, and growth of rye and sunflower.

Series	Number of plants	Silica source	Shoots		Entire plant
			Dry weight in grams	Silica p.p.m. fresh weight	Silica p.p.m. fresh weight
		50 p.p.m. soluble			
	9	Si in Crystalite	3.50	2950
	9	Kaolin	3.96	2450	3290
	9	Sandy loam	3.75	2130	2350
	9	Quartz sand	3.95	290	300
	3	Kaolin	3.46	1750	2220
	3	Sandy loam	2.94	1230	1890
	3	Quartz sand	3.54	220	210
	6	Serpentine soil plus water	3.14	2840
	6	Serpentine soil plus nutrient	9.21	1670

Series C4 (rye) and CC4 (sunflower) were grown for 70 days during summer, and were supplied with nutrient solution B at pH 5.5. Series CC6 (sunflower) was grown in serpentine soil for 42 days during spring. Plants of each treatment within a series were pooled before analysis.

TABLE 5. Influence of plants on dissolution of quartz sand at different pH values. The quantities are an average of readings made at seven consecutive twelve-hour intervals.

Series	Plant and control	pH			
		7.1	5.3	4.6	3.8
		p.p.m. dissolved silica			
B5	rye	2.4	2.2	2.2	1.5
BB5	sunflower	2.3	1.8	1.6	1.1
B5, BB5	control (no plants)	.8	.7	.7	.6

greater silica-absorbing capacity, than under sunflower.

Although data presented thus far have shown that soluble silica is absorbed freely, no definite conclusion could be drawn concerning the possible entry of less soluble or colloidal silica into the roots. Nanji and Shaw (1925) reported that silica may be taken up as colloidal silicic acid. On the other hand, Frey-Wyssling (1930), Ayres (1936), and Viehoveer and Prusky (1938) have stated, admittedly without much experimental evidence, that silica probably is absorbed as the soluble silicate ion, or as soluble silicic acid.

In series C2, CC2 and C3, plants were grown in collodion sacs, some of which separated bentonite or kaolin from the roots (table 6). In the latter cultures even greater amounts of silica were absorbed than in

cultures where roots were in actual contact with the clay materials, probably owing to the somewhat greater quantity of clay in the culture arrangement. As in series B5 and BB5, both rye and sunflower caused a slight though recognizable increase in silica solubility, irrespective of whether the roots were in contact with the clays. From the data of table 6, four conclusions seem possible and pertinent: (1) only soluble silica (that which passed a collodion membrane), and not colloidal silicic acids or siliceous particles, was taken up; (2) roots of both species secreted a substance which dissolved silica; (3) this substance was molecular rather than colloidal or enzymatic in character, since it passed freely through a collodion membrane; and (4) this substance was effective both when roots were in contact with clay and when they were separated from it.

Effect of carbon dioxide and nitrogen on solubility of siliceous materials.—Carbonic acid suggested itself as a root secretion which would pass a collodion membrane and which might dissolve silica from soil minerals. Treatment of various soils and siliceous materials with carbonic acid brought about an increase in soluble silica in all cases (table 7). Greater increases were obtained from bentonite and serpentine soil than from kaolin, quartz sand, or Lakewood soil. Materials containing a high percentage of quartz (sand and Lakewood soil) yielded only small amounts of initially soluble silica and showed only slight increases in silica solubility. Addition of sodi-

TABLE 6. Absorption and dissolution of silica by rye and sunflower from kaolin and bentonite suspensions.

Series ^a	Arrangement	Plants	Region tested	Age of cultures and controls in days		P.p.m. silica in fresh shoots
				6	42	
				P.p.m. dissolved silica in liquids		
C2, CC2	650 ml. of kaolin plus nutr. soln. outside sac; water only inside	0	inside sac	7.0	10.2
C2	same	rye	"	7.0	11.3	1190
CC2	same	sunflower	"	7.8	11.3	380
C2, CC2	water only outside sac; 350 ml. of kaolin plus nutr. soln. inside	0	outside sac	7.4	7.4
C2	same	rye	"	7.8	8.6	810
CC2	same	sunflower	"	7.8	7.8	310
C3	650 ml. of bentonite plus nutr. soln. outside sac; water only inside	0	inside sac	8.2	12.2
C3	same	rye	"	9.0	16.0	1120
C3	water only outside sac; 350 ml. bentonite plus nutr. soln. inside	0	outside sac	3.1	10.2
C3	same	rye	"	3.7	12.2	320

* The plants, grown at pH 5.5 in 350 ml. collodion sacs containing suspensions or water as indicated, were suspended in liter beakers containing the indicated suspensions or water.

TABLE 7. *Series DDD. Effect of nitrogen and carbon dioxide gases on dissolution of silica from liquid mixtures of certain clays, minerals, and soils. Duplicate series of the soil substances were aerated simultaneously with the gases for 4 days, then the order of gas treatment was reversed so that samples treated originally with carbon dioxide were given nitrogen, and vice versa.*

Mineral, clay or soil Kind	Gms. in 100 gms. of mixture	Liquid mixed with clay soil, etc.	Gas (original order)	Days treated		Final pH	Gas (reverse order)	Days treated	
				0	4			4	Final pH
Bentonite	3.7	nutr. soln. B	N ₂	7.7	19.1	7.1	CO ₂	24.6	5.7
			CO ₂	7.7	28.5	5.4	N ₂	23.8	7.6
Kaolin	15.0	nutr. soln. B	N ₂	4.7	11.4
			CO ₂	4.7	13.0
Kaolin	28.6	dist. water	N ₂	5.1	5.8	5.4	CO ₂	8.3	4.9
			CO ₂	5.1	6.1	4.9	N ₂	7.0	5.4
Kaolin	28.6	M/1 NaCl	N ₂	5.1	5.8	5.3
			CO ₂	5.1	6.1	4.8
Kaolin	28.6	M/1 CaCl ₂	N ₂	5.1	6.1	4.7	CO ₂	10.2	4.3
			CO ₂	5.1	7.0	4.3	N ₂	9.6	4.7
Quartz sand	44.4	dist. water	N ₂	2.3	2.7	6.8	CO ₂	5.1	4.5
			CO ₂	2.3	3.1	4.7	N ₂	3.5	6.7
Quartz sand	44.4	M/1 NaCl	N ₂	2.3	2.7	6.7	CO ₂	5.1	4.4
			CO ₂	2.3	3.5	4.7	N ₂	3.5	6.7
Lakewood soil	44.4	dist. water	N ₂	3.1	3.2	6.0	CO ₂	8.3	5.4
			CO ₂	3.1	3.5	5.4	N ₂	5.8	5.7
Serpentine soil	44.4	dist. water	N ₂	13.0	13.4	8.3	CO ₂	16.0	6.6
			CO ₂	13.0	26.2	6.4	N ₂	8.3	8.1

um chloride to kaolin and quartz sand caused no significant difference in silica solubility, but a slight increase followed the addition of calcium chloride to kaolin. A minor increase was noted also if nutrient B was utilized in place of water for the liquid phase of the kaolin mixture. Increases produced by nitrogen gas apparently were associated with the mechanical agitation of the bubbling gas.

When the gas treatments were reversed, so that samples which originally had received carbon dioxide were given nitrogen, and vice versa, soluble silica increased in the previously nitrogen-treated samples, but remained relatively constant in the previously carbon dioxide-treated ones. The increased solubility of carbon dioxide-treated samples was associated always with an increase in hydrogen-ion concentration.

DISCUSSION.—The literature reveals no general agreement as to the solubility of silica (Seidell, 1940; Mellor, 1940). Many factors, such as particle size, freshness of fracture, quantity present, salts, soil isoelectric complex, pH, and possibly enzymes influence its solubility at a given temperature. Nutting (1932, 1937, 1942) stated that pure silica obtained from bentonite or silica gel has a solubility in distilled water of 180 p.p.m. King (1938) indicated that the crystalline forms of silica were soluble to the extent of from 40 to 140 p.p.m. Correns (1940) gave a high value for the solubility of silicic acid gel, but Denny, Robson, and Irwin (1939) obtained only 27 p.p.m. dissolved silica from quartz and 7 p.p.m. from kaolin.

Lucas and Dolan (1939) evaluated several factors which contribute to the quartz solubility values they secured (50 to 100 p.p.m. of silica). They pointed out that other minerals present in quartz as impurities seriously interfere with the determination. The lowest value for the solubility of quartz has been reported by Titus (1937). After standing two months in water of approximately pH 7, quartz yielded only 0.5 p.p.m. of soluble silica. Evidence obtained in the present study supports the conclusion of Titus. The comparatively large amounts of silica dissolved from various soil materials in series DDD and absorbed by plants in series C4 and CC4 apparently originated directly from minerals other than quartz.

The criterion used to distinguish soluble from insoluble silica is a matter of controversy and has contributed to the general disagreement on silica solubility. Erbe (1935) has determined the particles of silicic acid to be between 40 and 50 millimicrons in diameter. These colloidal particles, by gravimetric methods of analysis, might erroneously be included in the soluble fraction. In this paper soluble silica was determined as that form of dispersed silica which forms silico-molybdic acid (Denny, Robson, and Irwin, 1939). The method, however, does not differentiate between dissolved silica and silicates (Titus, 1937).

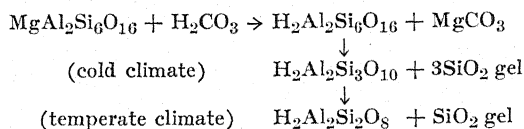
A number of workers have reported on the effect of pH on silica solubility. Correns (1940) found that silica dissolved from potassium feldspar was at a minimum between pH 6 and 7, whereas silicic

acid gel was least soluble at pH 3. Nutting (1932, 1943) stated that silica of clays and of many other minerals was least soluble at neutrality, and that the presence of even a trace of either an acid or a base increased its solubility. Mattson and Gustafsson (1937) found that silica of a podzol soil had a minimum solubility between pH 8 and 9. They observed that silica was carried into solution by the sesquioxides, resulting in an apparent increase in its solubility with an increase in hydrogen-ion concentration. Mann and Barnes (1940) noted that when the pH value of a light sandy loam was above 4.0, there was an almost constant amount of dissolved silicic acid in the drainage water; when the pH went down to 3.3, the proportion was doubled. Denny, Robson and Irwin (1939) found that minimum solubility of silica from both granite powder and asbestos wool occurred between pH 9 and 10, there being an increase on either side of this low. With respect to quartz powder, however, they found that its solubility increased rather steadily from pH 4 to 9.

Only minute quantities of silica were dissolved and absorbed from quartz sand at either high hydrogen-ion concentrations or neutrality (series B5 and BB5). However, large quantities of silica were absorbed from soil and soil materials of acidic reaction that contained silicates (series C4 and CC4). When the reaction of an aqueous mixture of siliceous materials was made more acidic by means of carbon dioxide (series DDD), the solubility of silica was increased most in those materials containing appreciable amounts of silicates. The slight increase in the solubility of quartz sand probably should be attributed to silicates present as impurities.

The explanation for the effect of certain salts on the solubility of silica may lie in part in their effect upon pH. In series DDD, sodium chloride, a neutral salt, was shown to be without effect on the solubility of silica in quartz sand and kaolin in the presence of nitrogen and carbon dioxide gases. However, calcium chloride, a somewhat acidic salt, caused a slight increase in both pH and in dissolved silica from kaolin under the same conditions. It is apparent, therefore, that one effect of a moderately high hydrogen-ion concentration (increased solubility of silica in soil silicates) is quantitatively more important than its other effect (decreased rate of absorption of soluble silica) in accounting for the amount of silica taken up by plants from many soils.

The exact manner in which carbonic acid brings about solution of silica from soil minerals remains obscure. Any hypothesis must take into account the dynamic nature of soil, the constant shifting in equilibria of reactions, and the atomic substitutions taking place. Natural weathering agents slowly decompose the minerals and leach out the more soluble compounds. Decomposition of a silicate may be initiated by an acid such as carbonic:



It is probable that a definite fraction of the liberated "silica gel" or silicic acid ($x\text{SiO}_2 \cdot y\text{H}_2\text{O}$) is at least temporarily in solution and is capable of passing a plant membrane.

Another potential source of soluble silica is suggested by the widespread occurrence of minerals which during decomposition might yield unstable sodium or potassium hydrogen silicate. Of the silicates, only those of the alkali metals are soluble to an appreciable extent. Little is known concerning either the existence or the solubility of naturally occurring organic silicates (Nanji and Shaw, 1925; Nutting, 1932; Viehoveer and Prusky, 1938).

Although the present study has indicated that root-secreted carbonic acid may bring into solution some silica or silicate from soils, insufficient data prevent making the claim that carbonic acid is the sole or even primary agent effecting solution. Graham (1941) found that certain colloidal organic acids were effective in the weathering of calcium from anorthite, though he did not state their effect upon silica. King and Davidson (1933) suggested the existence of an enzyme, silicatase, from their studies on diatoms. Albrecht, Graham, and Ferguson (1939) found that soybeans dissolved silica from a clay of extreme mineralogical stability. They concluded that carbonic acid was insignificant, compared with other plant agents, in rendering silica soluble. The writer, however, obtained results to the contrary with different soil materials. Carbonic acid secreted by roots, supplemented by that from soil microorganisms, most probably is an important silica-dissolving agent. Although only small amounts of this acid are secreted at any time and consequently only small increases in dissolved silica are detected, over any considerable period significant quantities are dissolved and absorbed. Accumulation from a dilute solution may be favored by the fact that certain ions and salts move from one clay particle to the next, thus obviating the necessity of immediate contact between the roots and the ions and salts (Albrecht, Graham, and Shepard, 1942).

SUMMARY

Through the employment of water, sand, and synthetic medium culture techniques, the influence of pH, silicon concentration, and silicon source on both growth and silicon absorption by rye and sunflower was studied. Silicon, supplied as soluble sodium silicate, was absorbed by both species at all seasons in approximate proportion to its concentration in the nutrient solution. At a high external concentration (450 p.p.m. of silicon) the plants accumulated silicon primarily in their roots; lower concentrations (150 p.p.m. of silicon and less) favored accumulation in the shoots, especially in the leaves. This relationship held for plants grown both in

liquid and in solid media. No plausible explanation for these distributions was obtained from data on transpiration rates and on solubility of silicon within the plants. The greater, though variable, portion of silicon in the plants occurred in an insoluble form. Of the two species, rye always took up more silicon.

Soluble silicon at high concentration was not toxic to the plants. Under some cultural and climatic conditions, growth was improved by the presence of silicon, but under other conditions no such improvement resulted.

Within a pH range of 3.6 to 7.1, maximum absorption of silicon (supplied in a soluble form) occurred at approximate neutrality. Considerable quantities were absorbed also at a pH value as low as 3.6. With respect to growth, the effect of pH varied with the season. During the growing season (spring) a pH value of 7.1 produced maximum growth of both species, whereas during the winter,

yield was best at pH 4.6. A pH of 7.1 during the latter season was toxic for rye.

Data obtained from an arrangement whereby roots were separated from clay suspensions by a collodion membrane indicated that no colloidal silicon was absorbed and that the roots secreted a silicon-dissolving substance of molecular dimensions. Evidence suggested that this root secretion was carbonic acid, which was shown to be capable of dissolving appreciable quantities of silicon from various soils containing silicates, but not from quartz sand. It was concluded that under natural conditions silicon probably is absorbed by plants principally as temporarily-soluble silicic acid and as soluble silicates arising from the decomposition of complex silicates. The results of this study re-emphasize and broaden the role plants serve in the weathering of rocks and in the building of a soil.

DEPARTMENT OF BOTANY,
UNIVERSITY OF PENNSYLVANIA,
PHILADELPHIA, PENNSYLVANIA

LITERATURE CITED

- AYRES, ARTHUR. 1936. Effect of age upon the absorption of mineral nutrients by sugar cane under field conditions. *Jour. Amer. Soc. Agron.* 28: 871-886.
- ARRHENIUS, O. 1929. Soil acidity. Plant growth and its practical application. *Proc. Int. Cong. Plant Sci.*, Ithaca. 1: 53-54.
- ARNON, D. I. 1937. Ammonium and nitrate nitrogen nutrition of barley at different seasons in relation to hydrogen-ion concentration, manganese, copper, and oxygen supply. *Soil Sci.* 44: 91-122.
- . 1939. Effect of ammonium and nitrate nitrogen on the mineral composition and sap characteristics of barley. *Soil Sci.* 48: 295-307.
- ALBRECHT, W. A., E. R. GRAHAM, AND C. E. FERGUSON. 1939. Plant growth and the breakdown of inorganic soil colloids. *Soil Sci.* 47: 455-458.
- , ———, AND H. R. SHEPARD. 1942. Surface relationships of roots and colloidal clay in plant nutrition. *Amer. Jour. Bot.* 29: 210-213.
- BYERS, H. G. 1935. Selenium occurrence in certain soils in the United States, with a discussion of related topics. *U. S. Dept. Agric. Tech. Bull.* No. 482.
- COOPER, H. P. 1930. Ash constituents of pasture grasses, their standard electrode potential and ecological significance. *Plant Physiol.* 5: 193-215.
- CORRENS, C. W. 1940. Die chemische Verwitterung der Silikate. *Naturwissenschaften* 28: 369-376.
- DENNY, J. J., W. D. ROBSON, AND D. A. IRWIN. 1939. The prevention of silicosis by metallic aluminum. *Can. Med. Assoc. Jour.* 40: 213-228.
- ERBE, F. 1935. Die Bestimmung der Teilchengröße von Kieselsäure in einem Kieselsäure-Glyzerosol. *Kolloid Zeit.* 73: 1-14.
- FREELAND, R. O. 1936. Effect of transpiration upon the absorption and distribution of mineral salts in plants. *Amer. Jour. Bot.* 23: 355-362.
- . 1937. Effect of transpiration upon the absorption of mineral salts. *Amer. Jour. Bot.* 24: 373-374.
- FREY-WYSSLING, A. 1930. Über die Ausscheidung der Kieselsäure in der Pflanze. *Ber. Deutsch. Bot. Ges.* 48: 179-183.
- GERMAR, B. 1934. Über einige Wirkungen der Kieselsäure in Getreidepflanzen, insbesondere auf deren Resistenz gegenüber Mehltau. *Zeit. Pflanzenernähr., Düng. Bodenk.* A35: 102-115.
- GRAHAM, E. R. 1941. Colloidal organic acids as factors in the weathering of anorthite. *Soil Sci.* 52: 291-295.
- HAMPTON, H. E., AND W. A. ALBRECHT. 1943. Nodulation modifies nutrient intake from colloidal clay by soybeans. *Proc. Amer. Soc. Soil Sci.* 8: 234-237.
- HOAGLAND, D. R. 1937. Some aspects of the salt nutrition of higher plants. *Bot. Rev.* 3: 307-334.
- , AND J. C. MARTIN. 1923. A comparison of sand and solution cultures with soils as media for plant growth. *Soil Sci.* 16: 367-388.
- , AND D. I. ARNON. 1941. Present concepts of ion availability in plant nutrition. Physiological aspects of availability of nutrients for plant growth. *Soil Sci.* 51: 431-444.
- KARDOS, L. T., AND J. S. JOFFE. 1938. The preparation, composition, and chemical behavior of the complex silicates of magnesium, calcium, strontium, and barium. *Soil Sci.* 45: 293-307.
- KING, E. J. 1938. Solubility of silica. *Lancet* 234: 1236-1238.
- , AND V. DAVIDSON. 1933. The biochemistry of silicic acid. IV. Relation of silica to the growth of phytoplankton. *Biochem. Jour.* 27: 1015-1021.
- LOOMIS, W. E., AND C. A. SHULL. 1937. Methods in plant physiology. McGraw-Hill Book Co., New York.
- LUCAS, C. C., AND M. E. DOLAN. 1939. The solubility of quartz and silicates. *Can. Med. Assoc. Jour.* 40: 126-134.
- LUNDEGARDH, H. 1934. Mineral nutrition of plants. *Ann. Rev. Biochem.* 3: 485-500.
- MACINTYRE, W. H., W. M. SHAW, AND J. B. YOUNG. 1925. The role of silica in counteracting magnesia-induced toxicity. *Soil Sci.* 19: 331-341.
- MANN, H. H., AND T. W. BARNES. 1940. Studies of soil after fifty years of wheat and barley cropping, especially of soil made acid with sulfate of ammonia. *Jour. Agric. Sci.* 30: 345-386.
- MATTSON, S., AND Y. GUSTAFSSON. 1937. The laws of soil colloidal behavior. XIX. The gel and the sol complex of soil formation. *Soil Sci.* 43: 453-475.
- MELLOR, J. W. 1940. A comprehensive treatise on inor-

- ganic and theoretical chemistry. 6:141. Longmans, Green and Co., London.
- NANJI, D. R., AND W. S. SHAW. 1925. The role of silica in plant growth: its assimilation and physiological relation to phosphoric acid. Jour. Soc. Chem. Ind. 44:1T-6T.
- NUTTING, P. G. 1932. The solution and colloidal dispersion of minerals in water. Jour. Washington Acad. Sci. 22:261-267.
- . 1937. The study of bleach clay solubility. Jour. Franklin Inst. 224:339-362.
- . 1942. A study of ionic adsorption in solutions of silica and alumina. Jour. Washington Acad. Sci. 32:117-122.
- . 1943. The action of some aqueous solutions on clays of the montmorillonite group. U. S. Geol. Survey, Prof. Paper No. 197F, pp. 219-233.
- OSTERHOUT, W. J. V. 1936. The absorption of electrolytes in large plant cells. Bot. Rev. 2:283-315.
- RALEIGH, G. J. 1939. Evidence for the essentiality of silicon for growth of the beet plant. Plant Physiol. 14:823-828.
- RICHARDSON, W. D. 1920. The ash of dune plants. Sci. n. s. 51:546-557.
- SCHMIDT, OSWALD. 1936. Die Mineralstoffaufnahme der höheren Pflanze als Funktion einer Wechselbeziehung zwischen inneren und äusseren Faktoren. Zeit. Bot. 30:289-334.
- SCHWARTZ, M. C. 1934. Colorimetric determination of silica in boiler water. Ind. Eng. Chem., Anal. Ed., 6:364-367.
- SEIDELL, A. 1940. Solubilities of inorganic and metal organic compounds. 1:1488. Van Nostrand Co., Inc., New York.
- STEWART, F. C. 1935. Mineral nutrition of plants. Ann. Rev. Biochem. 4:519-544.
- TITUS, A. C. 1937. Silica and silicate solubilities. Jour. Ind. Hyg. Toxicol. 19:138-145.
- TRELEASE, S. F., AND H. M. TRELEASE. 1938. Selenium as a stimulating and possibly essential element for indicator plants. Amer. Jour. Bot. 25:372-380.
- VIEHOEVER, ARNO, AND S. C. PRUSKY. 1938. Biochemistry of silica. Amer. Jour. Pharmacy 110:1-22.
- WAGNER, FRITZ. 1940. Die Bedeutung der Kieselsäure für das Wachstum einiger Kulturpflanzen, ihren Nährstoffhaushalt und ihre Anfälligkeit gegen echte Mehltäupilze. Phytopath. Zeit. 12:427-479.
- WHERRY, E. T. 1932. Ecological studies of serpentine-barren plants. I. Ash composition. Proc. Pennsylvania Acad. Sci. 6:32-38.
- WRIGHT, K. E. 1939. Transpiration and the absorption of mineral salts. Plant Physiol. 14:171-174.

GENETICS OF GLOMERELLA. III. CROSSES WITH A CONIDIAL STRAIN¹

S. J. P. Chilton, G. B. Lucas, and C. W. Edgerton

IN A previous paper, Lucas, Chilton, and Edgerton (1944) reported that ascospores isolated from a plus strain of a culture of *Glomerella* originally obtained from *Ipomoea* gave rise to the parent strain and to several new strains differing in such characters as the production of conidia, the formation of perithecia, and self-fertility. Some of these strains formed lines of fertile perithecia when they came in contact with the parental strain and with each other. In a second paper (Edgerton, Chilton, and Lucas, 1945), evidence was presented to show that a fertilization takes place between strains when the ridge of perithecia develops as they come in contact.

Among the isolates developing from ascospores of the plus strain, which were entirely unlike any of the plus or minus strains, were certain ones which did not produce perithecia but did produce conidia. These isolates were not identical and for convenience are at present being placed together in a group designated as the conidial type. The individual ones which are receiving attention in this paper have been designated as conidial A, conidial B, and conidial C.

The results presented in this paper are based mainly on work with the conidial A strain, called the heavy conidial strain in a preceding paper. The conidial A strain produces large pink spore masses on the surface of oatmeal agar, and in all ways is very similar to the non-ascogenous *Colletotrichum* and *Gloeosporium* forms which are well-known from many host plants.

When the conidial A strain is grown in a plate

with either a plus or a minus strain, a ridge of perithecia develops on the line of contact. This ridge develops slowly as compared to those which develop between plus and minus strains, and while in some cases the number of perithecia is rather limited, those which do form are well-developed and full of normal asci.

Previous to the present investigations, no one seems to have reported in *Glomerella* the presence of perithecia on the line of contact between perithecial and non-perithecial cultures or between conidial and non-conidial cultures. This paper reports the results obtained from crossing the conidial A strain with certain plus and minus strains.

STRAINS STUDIED.—Crossing tests were made with four strains: plus A, minus A, minus B, and conidial A. Besides these, two other conidial strains appeared in the progeny of certain crosses. These six strains are briefly described.

Plus A. Originally from *Ipomoea*. Perithecia in glomerate masses. Ascospores well developed. Conidia not observed. Ascospores when isolated produce colonies of both plus and minus strains. Very rarely colonies of conidial A have developed from ascospores.

Minus A. From ascospores of the plus A strain. Perithecia scattered over surface of medium, ordinarily sterile, only an occasional one producing a few ascospores. Conidia very few. Ascospores on germination produce only minus A colonies. (A similar strain from a plus A strain from okra, *Hibiscus esculentus*, also included in one test.)

¹ Received for publication May 28, 1945.

Minus B. From an ascospore of the plus A strain. Similar to the minus A strain except the perithecia are filled with well-developed asci and ascospores. Ascospores on germination produce only minus B colonies.

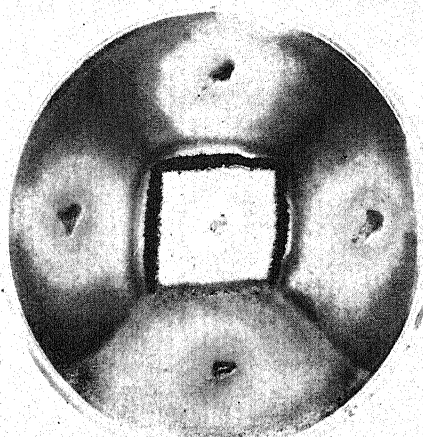


Fig. 1. Petri dish with four cultures of the conidial B strain mated with a plus A strain. One strain (below) produced only a few scattering perithecia on line of contact, while others produced dense ridges of perithecia.

Conidial A. From an ascospore of the plus A strain. Isolates which are similar and probably identical have also appeared as sectors in plate cultures of the plus A strain. Cultures produce no perithecia but are covered with numerous pink masses of conidia. (A similar strain from a plus A strain from okra also included in one test.)

Conidial B. From ascospores derived from several of the crosses. Conidial masses forming on oatmeal agar are smaller than those produced by the conidial A strain. Indistinguishable from the conidial A strain on bean agar. As all cultures grouped together here have not reacted alike when mated with other strains, this may be a complex rather than a single strain. For example, when mated with the plus A strain some cultures produce a dense ridge of perithecia while others produce only a few scattering perithecia (fig. 1).

Conidial C. From ascospores derived from one of the crosses. Similar to conidial A but cultures contain small brown sclerotium-like bodies suggestive of rudimentary perithecia.

METHODS.—The methods were in general the same as those described in previous papers. Plantings of two strains were made in a petri dish and the colonies allowed to grow together. When the ridge of perithecia developed on the line of contact, individual ascospores were picked up with a micromanipulator and transferred to tubes of culture medium. The cultures developing from the ascospores were compared

and identified as to strain. To analyze individual asci all eight spores were isolated from an ascus and grown separately. Since the asci in a perithecium are held together by the ascogenous hyphae, it was possible to analyze several asci from individual perithecia. Ordinarily when cultures were obtained from five ascospores from an ascus it was possible to tell the strain to which the missing ones belonged.

In order to designate crosses and cultures, the following system was used. The original isolates were given the symbols Ip or O denoting their origin either from *Ipomoea* or from okra. In the crosses, an arbitrary number was assigned to each cross from which ascospores were isolated. Asci from which individual ascospores were isolated and cultured were designated by capital letters, and the individual spores from each ascus were numbered from one to eight. Thus 5-K-3 indicates that the culture was isolated from cross 5 and came from ascus K. Where ascospores were isolated at random, numbers were given to the cultures, i.e., 5-16. As examples of the system, 5-K-3-A-4 is the symbol for a culture from ascus A from the culture 5-K-3, and 5-K-3-16 for a culture from an ascospore isolated at random from 5-K-3. Small letters instead of numbers were used to designate cultures made from single conidia.

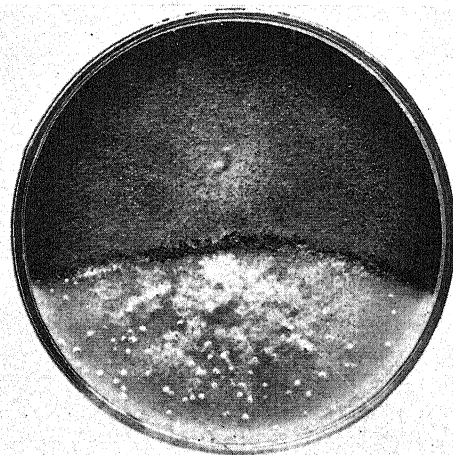


Fig. 2. Perithecial ridge on line of contact between plus A (above) and conidial A strains.

PLUS A AND CONIDIAL A.—Five different crosses were made between cultures of the plus A and conidial A strains. In each, the ridge of perithecia developed slowly on the line of contact (fig. 2). The asci which formed in these perithecia were noticeably longer than those produced by the plus A strain growing alone or those from other crosses.

The asci from the perithecial ridges were analyzed by picking up the single ascospores and, after culturing, identifying the strains that developed from them. The distribution of strains in 60 asci is presented in table 1. Of these asci, 44 had 4 spores each of the plus type and the conidial A strain, while 16

TABLE 1. Distribution of strains in 60 asci from 5 crosses between plus A and conidial A cultures of *Glomerella* from *Ipomoea*.

Cross number	Parents Plus A × conidial A	Number of asci in each segregation group		Total asci
		4 plus ^a 4 conidial A	8 conidial A	
7	5-K-3 × Ip-7-16-m.....	11	0	11
35	5-AA-4 × Ip-7-16-m.....	12	15	27
11	8-A-6 × 8-A-1	14	0	14
13	11-F-7 × 11-D-5	2	1	3
14	11-K-6 × 11-L-6	5	0	5
	Total asci	44	16	60

^a Since progeny tests were not made from all cultures to determine if they would produce the minus A strain, they are designated merely as plus. This also applies to the succeeding tables in this paper.

had 8 spores of the conidial A strain. Where several asci were analyzed from a single peritheciun, the spore distribution was either in the 4:4 ratio or in the 0:8 ratio but not in both. This indicates that the two ratios were determined by the genetic make-up of the nuclei which entered and fused in the asci.

It is assumed that the 4:4 ratio occurred in those asci into which a nucleus from the plus A strain and one from the conidial A strain entered and fused. If it is assumed that the 0:8 ratio occurred in those asci in which two nuclei of the conidial A strain fused, then the two strains would differ by one main factor (Aa) or a relatively closely-linked group of factors.

That the asci giving cultures of the two parent strains arose as a result of a fertilization of one strain by the other seems reasonably clear. The origin of the asci which gave only cultures of the conidial A strain is however not so clear. While these asci could have been formed either as a result of fertilization of one strain by the other with two nuclei of the conidial A strain pairing and entering the ascogenous hypha, or as a result of a stimulation of the conidial A strain to produce ascospores by the plus A strain, this does not preclude the possibility that some other explanation may be the correct one.

That 15 of the 16 asci with the 0:8 ratio came from a single cross may be significant. The parent plus A strain in this cross appeared to be slightly different from other plus A strains studied. When ascospores

of this strain were cultured, a few minus A isolates were obtained but most of the isolates were of the plus A strain.

MINUS A AND CONIDIAL A.—Four different crosses were made between cultures of the minus A and conidial A strains, three with the form from *Ipomoea* and one with the form from okra. The perithecial ridges on lines of contact were not as conspicuous as in some of the other crosses but the asci and ascospores were well-developed. The ascospores were isolated by picking up spores at random from crushed perithecia and by picking up individual ascospores from single asci.

The identity of the isolates from 245 ascospores is given in table 2. These were distributed among four strains or strain types; conidial A, conidial B, minus A, and a strain of the plus type in approximately a 1:1:1:1 ratio. If the conidial A and plus A strains differ by one factor (Aa) or a closely linked group of factors as indicated by the crosses described above, and if the plus A and minus A strains also differ by a single factor (Bb), which can be assumed from results presented in a previous paper (Edgerton, Chilton, and Lucas, 1945), the conidial A and minus A strains would differ by two factors (AaBb) and such a distribution would be expected. The four genotypes should appear in the crosses between the conidial A and minus A if the genes controlling the differences are not at the same locus. If they are at

TABLE 2. Strains from 245 ascospores from perithecia formed between conidial A and minus A strains of *Glomerella* from *Ipomoea* and okra.

Cross number	Parents	Number of ascospores of each strain				Total
	Conidial A × minus A	Conidial A	Minus A	Plus	Conidial B	
Ipomoea						
3	Ip-7-15 × 1-B-1	18	8	9	17	52
4	Ip-7-15 × Ip-7-38	9	3	4	8	24
5	Ip-7-16-m × 4-17	27	37	23	19	106
Okra						
19	O-55-m × O-1-21-13	13	22	12	16	63
	Total	67	70	48	60	245

TABLE 3. *Distribution of strains in 23 asci from the crosses between conidial A and minus A cultures of Glomerella.*

Number of asci in each segregation group					
4 conidial A 4 minus A	4 plus 4 conidial B	2 conidial A 2 minus A 2 plus 2 conidial B	4 conidial A 2 minus A 2 conidial B	8 minus A	Total asci
5	2	13	1	2	23

the same locus, the two parental strains only should appear in the cross.

Sufficient ascospores were isolated from each of 23 asci to determine the constitution of each. The distribution of the strains in these asci is shown in table 3. Five of the asci had four spores each of the minus A and conidial A strains, two asci had four spores each of a plus and the conidial B strains, and 13 asci had two spores of each of the four possible strains. The constitution of these 20 asci indicated that a fertilization between the strains had occurred. The 2:2:2:2 ratio found in 13 asci must have resulted from a segregation in the two nuclear divisions in the ascus and can be attributed to crossing-over. Both the 4:4 and the 2:2:2:2 ratios were found in the same perithecium. Statements could be made in regard to the asci with eight spores of the minus A strain similar to those used for the crosses between the plus A and conidial A strain where asci with eight spores of the conidial A strain were found. No satisfactory explanation can be given for the asci with four spores of the conidial A strain and two each of the conidial B and minus A strains.

Six of the plus cultures which were secured from individual ascospores from these crosses were allowed to mature and again to produce perithecia. Ascospores from these perithecia were isolated in order to determine if they were of the plus A strain; that is, if they would produce both plus and minus strains. From five of these, both plus and minus A strains were obtained. From one culture, however, no culture of the minus strain was obtained, although 1594 ascospores were isolated from it and its ascospore progeny. This culture which was given the symbol plus B was reported in a previous paper (Edgerton, Chilton, and Lucas, 1945).

MINUS B AND CONIDIAL A.—Four different crosses were made between cultures of the minus B and conidial A strains. The perithecial ridges on the lines of

contact appeared but little different from those between the minus A and conidial A strains. Ascospores were isolated in two ways, by picking up spores at random and by picking up the individual spores from single asci.

The identity of the isolates from 397 ascospores is given in table 4. These were distributed among five strains or strain types: conidial A, conidial B, conidial C, minus B, and a strain or strains of the plus type. In the main, the distribution conformed to the pattern found in the crosses between minus A and conidial A strains except for a large excess of isolates of the minus B strain and the presence of a few of the conidial C strain. It is possible that the large number of isolates of the minus B strain resulted from picking up ascospores near the perithecial ridges which may have been produced by the minus B parent. The minus B strain produces ascospores in great numbers and ascospores entirely of minus B origin could have developed at the edge of a perithecial ridge. No explanation is available for the occurrence of the isolates of the conidial C strain. Since all four asci came from one perithecium, it is possible that a nucleus genetically different from the plus was included in the fusion nuclei in these asci. Just how this occurred is unknown.

The constitution of 48 asci was obtained by picking up individual ascospores from single asci. The distribution of the various strains is shown in table 5. In the main, the distribution in the different segregation groups was similar to that obtained in the crosses between minus A and conidial A strains. If the 14 asci containing only spores of the minus B strain and the four asci with the conidial C strain are not considered, the data indicate that the conidial A and minus B strains differ by two factors (AaBb') or two groups of factors so closely linked that they did not separate in the limited progeny studied. The evidence seems to indicate that the factor for self-

TABLE 4. *Strains from 397 ascospores from perithecia formed between conidial A and minus B strains of Glomerella from Ipomoea.*

Cross number	Parents Conidial A × minus B	Number of ascospores of each strain					Total
		Conidial A	Minus B	Plus	Conidial B	Conidial C	
8	Ip-7-16-m × Ip-7-35-2-6	11	11	13	9	..	44
10	8-A-3 × 8-A-5	10	70	80
15	8-A-3 × 8-A-1	19	46	31	19	13	128
36	Ip-7-16-m × 17-F-5	31	54	32	28	..	145
	Total	71	181	76	56	13	397

TABLE 5. Distribution of strains in 48 asci from crosses between conidial A and minus B strains of *Glomerella*.

Number of asci in each segregation group					
4 conidial A 4 minus B	4 plus 4 conidial B	2 conidial A 2 minus B 2 plus 2 conidial B	4 minus B 4 conidial C	8 minus B	Total asci
6	3	21	4	14	48

fertility in the minus B strain is linked with the factor or factors responsible for the minus characters since they segregated together.

Ascospores were isolated from five of the plus strains obtained in the crosses to determine if they would produce the minus A strain. All of these produced a plus strain and the minus A strain which demonstrated they were of the plus A strain.

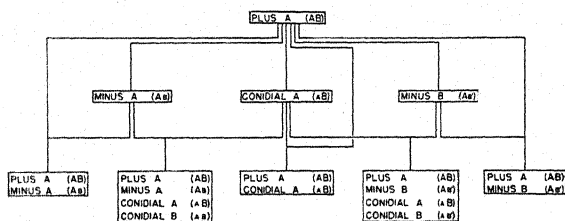


Fig. 3. Diagram illustrating the genetic make-up of the different strains as shown by the various crossing tests.

DISCUSSION.—To make the results reported in this and preceding papers more easily understood, a diagram has been prepared (fig. 3). In this, the more common strains obtained from the original plus A strain and the strains from the various crosses have been designated by letters in an attempt to show the genetic make-up of each strain as indicated by the data from the crosses. The letters which have been used do not necessarily represent single genes. They may include a number of genes closely enough linked so that they did not separate in the relatively small populations studied. In the diagram, the conidial C strain was excluded for the reason given above, and the asci in the crosses giving eight cultures of one parent strain were disregarded. The evidence from the crosses indicates that the minus A, minus B, and conidial A strains are genetic entities differing from the plus A strain from which they came. Just how they arose is not clear. The phenomenon is similar in some respects to that reported by Lindegren (1934) in his paper on the inheritance of the characters tan and normal in *Neurospora*, and could be interpreted as due to mutation. However, until further studies are made of the nature of the difference between the plus A strain which gives such a high percentage of minus A strains and the plus B strain which has given none in a rather large number of ascospore cultures derived from it and its progeny, it would seem wise to postpone speculation as to the method by which these strains arise.

Some interesting problems related to or associated with the production of conidia in the genus *Glomerella* have been brought out or are suggested by the results reported in the present paper.

In picking up ascogenous cultures of *Glomerella* from nature, it is found that they may or may not produce conidia. Some cultures produce conidia in such abundance that the surface of the medium is covered with pink spore masses. On the other hand, some cultures with perithecia in abundance produce no conidia. It seems evident that the ability to produce conidia in culture is an inherited characteristic and that the factors responsible are distinct from the ones responsible for the formation of perithecia.

Granting that the production of conidia is determined by genetic factors, then it becomes important to explain how after numerous transfers, cultures sometimes seem to lose their ability to produce conidia. This loss of sporulation has been reported by numerous investigators and has usually been attributed to running out or to mutations. It is recognized that cultures can be maintained if frequent transfers are made from growing hyphal tips or from newly formed conidia. On the other hand, if transfers are only made from old mycelium, deterioration of the cultures with the loss of sporulation is frequently observed. Reasoning from the observations reported by various workers not only with *Glomerella* but also with other fungi, the evidence indicates that the loss of sporulation may be due to mutation or possibly to a series of mutations, resulting from chromosome aberrations or gene changes which may have been brought about by malnutrition, staling products in the culture medium, or from some other cause. It, however, does not necessarily follow that the absence of conidia in these old cultures has the same explanation as the absence of conidia in some of the ascogenous cultures of *Glomerella* picked up in nature. That this is not the case is suggested by results presented in this paper where conidial cultures were obtained as sectors in non-conidial cultures and from ascospores from non-conidial cultures.

In all the crosses made between the conidial A strain and strains of the plus and minus types in which conidia were not observed, not a single culture was obtained in the progeny which produced both conidia and perithecia. As ascogenous cultures of *Glomerella* which also produce conidia in abundance are commonly obtained from various host plants, it is not quite clear why similar cultures were not obtained from crosses between a non-conidial asco-

genous culture and a conidial non-ascogenous culture.

The origin of the non-conidial ascogenous cultures which have been reported in the past by various investigators is somewhat obscure. Presumably, they were picked up on host material on which conidia were present. Whether they actually came from conidia was, however, not determined with certainty. That such cultures can come from conidia was indicated by some recent isolation tests. During the summer of 1945, anthracnose lesions were common on stems of cowpeas and soybeans in south Louisiana. From material containing both ascospores and conidia, the fungus was cultured by picking up spores. As the ascospores were decidedly curved and the conidia were straight, there was only a slight chance of confusing the two. Cultures were obtained from eight to ten ascospores and from a similar number of conidia from each of two lots. All cultures which were obtained produced on oatmeal agar perithecia of the plus type but no conidia. This suggests that some of the cultures which are considered to be non-conidial might on the proper substratum produce conidia.

For many of the species of *Colletotrichum* and *Gloeosporium* which have been studied in the past, no ascogenous stage has been reported and from most forms only conidial cultures have been obtained. It is possible that, as in the conidial A strain, perithecia would be produced if mated with the appropriate strain. On the other hand, it is possible that some of these have such a genetic constitution

that they will not produce perithecia either alone or when mated with any strain.

SUMMARY

A conidial strain was obtained from a plus strain of a *Glomerella* from *Ipomoea*. This strain did not produce perithecia.

This conidial strain was crossed with strains of the plus and minus types. In the crosses between the plus A and the conidial A, perithecia formed rather slowly, but in the crosses between the conidial A strains and strains of the minus type, perithecia formed more quickly.

In the crosses between plus A and conidial A, ascospores produced cultures of the plus A and conidial A strains.

In the crosses between the conidial A and strains of the minus type, usually four different strains were obtained. Among the strains obtained was another conidial strain termed conidial B.

DEPARTMENT OF BOTANY,
LOUISIANA STATE UNIVERSITY,
BATON ROUGE, LOUISIANA

LITERATURE CITED

- EDGERTON, C. W., S. J. P. CHILTON, AND G. B. LUCAS. 1945. Genetics of *Glomerella*. II. Fertilization between strains. *Amer. Jour. Bot.* 32: 115-118.
LINDEGREN, C. C. 1934. The genetics of *Neurospora*. IV. The inheritance of tan versus normal. *Amer. Jour. Bot.* 21: 55-65.
LUCAS, G. B., S. J. P. CHILTON, AND C. W. EDGERTON. 1944. Genetics of *Glomerella*. I. Studies on the behavior of certain strains. *Amer. Jour. Bot.* 31: 233-239.

EVIDENCE FOR GENETIC VARIATION AMONG APOMICTICALLY PRODUCED PLANTS OF SEVERAL F₁ PROGENIES OF GUAYULE (PARTHENIUM ARGENTATUM) AND MARIOLA (P. INCANUM)¹

Reed C. Rollins²

It is almost axiomatic that the apomictic progeny of an individual plant should exactly duplicate this plant genetically and physiologically if purely mitotic cell divisions uncomplicated by somatic mutations preceded the formation of egg cells giving rise to this type of offspring. Actually, in guayule and mariola, most of the individuals of the apomictically produced progenies studied have conformed to this pattern of inheritance, but in nearly every instance there have been a few individuals which deviated to some degree. A small portion of these deviating maternal types were found to possess only about half the number of chromosomes present in the mother plant. These "haploid" plants, although maternal in their inheritance, deviate from the

mother plant because of the changed chromosome number, and because meiosis preceded the ultimate formation of the eggs which gave rise to them, thus providing a mechanism whereby genetic segregation could readily occur. However, variants without differences in chromosome number were also found within the apomictically produced portion of given progenies. It is this group of plants with which we are concerned. Variant plants of the latter type are potentially a menace to the uniformity of a commercial variety or strain of guayule where reproduction is largely by apomixis. Therefore, it is desirable to obtain some notion as to the frequency of occurrence of this type of variability. At the outset, it should be realized that the data presented below are not extensive enough to represent an accurate picture of the amount of variability occurring among apomictically produced plants of guayule as a whole. A much wider sample, including more diverse biological types, is needed before inclusive generaliza-

¹ Received for publication June 1, 1945.

² Much of this work was done while the author was on the staff of the Special Guayule Research Project, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture.

tions may be rightfully made upon this point. However, the general trend is clearly indicated.

MATERIAL AND METHODS.—Crosses between guayule and other species of *Parthenium* provided ideal material for a study of the apomictically produced portion of each progeny. In each cross, pollen was carefully controlled using the methods tested and described by Powers and Rollins (1945). Progenies of eleven crosses using guayule as the female have been studied where species of *Parthenium* other than guayule (*P. argentatum*) were used as the pollen parent. This facilitated the recognition of plants produced as a result of sexuality for they were all interspecific hybrids. Thus the sexually produced plants were readily separated from the apomictically produced portion of each progeny. Two progenies of mariola ♀ × guayule ♂ were also studied. Three $2n=36$ -chromosome "haploid" plants of guayule which reproduced largely by apomixis were used. Two of these came from wild sources and were from collections which were predominantly of the ± 72 -chromosome group. Bergner (1944), Stebbins and Kodani (1944), and Powers and Rollins (1945) have given the history of some plants of this type. They are haploid with respect to their immediate or recent progenitors, but are not haploids of the species. The third 36-chromosome plant used in these experiments was an apomictic daughter of one of the 36-chromosome plants described above. Six $2n=72$ -chromosome guayule plants were used. All six plants possessed two very small chromosomes in addition to the 72 of approximately equal size.³ Five of these plants were from cultivated strains, the other came from Texas. The two plants of mariola used were from wild sources and possessed $2n=54$ chromosomes.

The seeds were sprouted in a germinator and removed daily to be planted individually in 2½-inch unglazed pots. Plants taken from the germinator on a given date were kept together throughout the experiment. Comparisons were made only between plants of exactly the same age. Sterile pasteurized sand was used as a culture medium. Applications of one-fourth strength Hoagland's (1939) nutritive solution no. 1 were made daily to the plants throughout the course of the experiment. The pots were plunged into sawdust in flats in a uniform manner to prevent excessive moisture loss. The pots were frequently shifted about to equalize the possible effect of position in the flat upon the growth of the plants. As soon as the plants reached a size where the leaves began to touch those of adjacent plants, they were separated by uniform spacing. Thus the possible effect of crowding or shading upon plant growth was eliminated.

The total cultural treatment of the plants was aimed to produce a uniform environment so that deviations in growth rate, time of flowering, and phenotypical appearance occurring among plants

of the same age and progeny would not logically be attributable to environmental factors. The success of the procedures outlined above, in excluding to a large extent the differential effects of environment upon the growing plants, is shown by the fact that most of the plants in each age-group of each progeny were so nearly identical that they could not be distinguished from each other (fig. 5).

The progenies of guayule were studied repeatedly throughout the four-month period while the plants grew from seedlings to flowering. The mariola plants were similarly studied, but were not in flower at the end of four months. At the end of the first month a tentative classification of the progenies into strictly maternal types, which included a majority of the plants of each progeny, and deviating maternal types, was made. The latter included the plants in each age-group showing noticeable deviations of growth rate or phenotypical appearance from the uniform majority. The most striking differences observed were those of growth rate (as expressed by size) and the time of flowering. However, differences in habit and those of a minor morphological nature appeared to be correlated with size and time of flowering. The experiment did not run for a long enough period to obtain the fullest expression of such characteristics as habit of growth. At monthly intervals when the plants were two months, three months and four months old, the classification was rechecked. During the experiment, the trichome sizes of the deviating maternals were compared with the trichome sizes of the strict maternals in order to detect any plants which possessed a reduced ($1n$) chromosome number. Previous data (Rollins, 1944, and unpublished) showed that the trichomes of ca. 36-chromosome guayule plants are much shorter than those of the ca. 72-chromosome plants of the same strain. This method is effective in detecting ca. 36-chromosome plants among those of the ca. 72-chromosome level, but plants reduced from the 36-chromosome group have not been found in guayule, and it is not known what effect a further reduction in chromosome number would have upon trichome size.

At the conclusion of the experiment, each deviating maternal plant was paired with a strictly maternal one of the same age for further observation and comparison. The pollen size of each of the deviating maternals was compared with that of its strictly maternal partner. This provided a further check on the possibility of the deviating maternals having a reduced chromosome number. Bergner (1944), Powers (1945) and Powers and Gardner (1945) have shown that pollen size is correlated with chromosome number in guayule. The chromosome number of a portion of the paired plants was determined from aceto-orcein smears of root-tips. The method of Meyer (1945) was followed in the preparation, fixation and staining of the root-tips.

EXPERIMENTAL RESULTS.—The results of a careful classification of the apomictically produced portions of eleven F_1 progenies of guayule are presented in

³ Bergner (unpublished) has called the small chromosomes of the type present in these plants, miniature chromosomes.

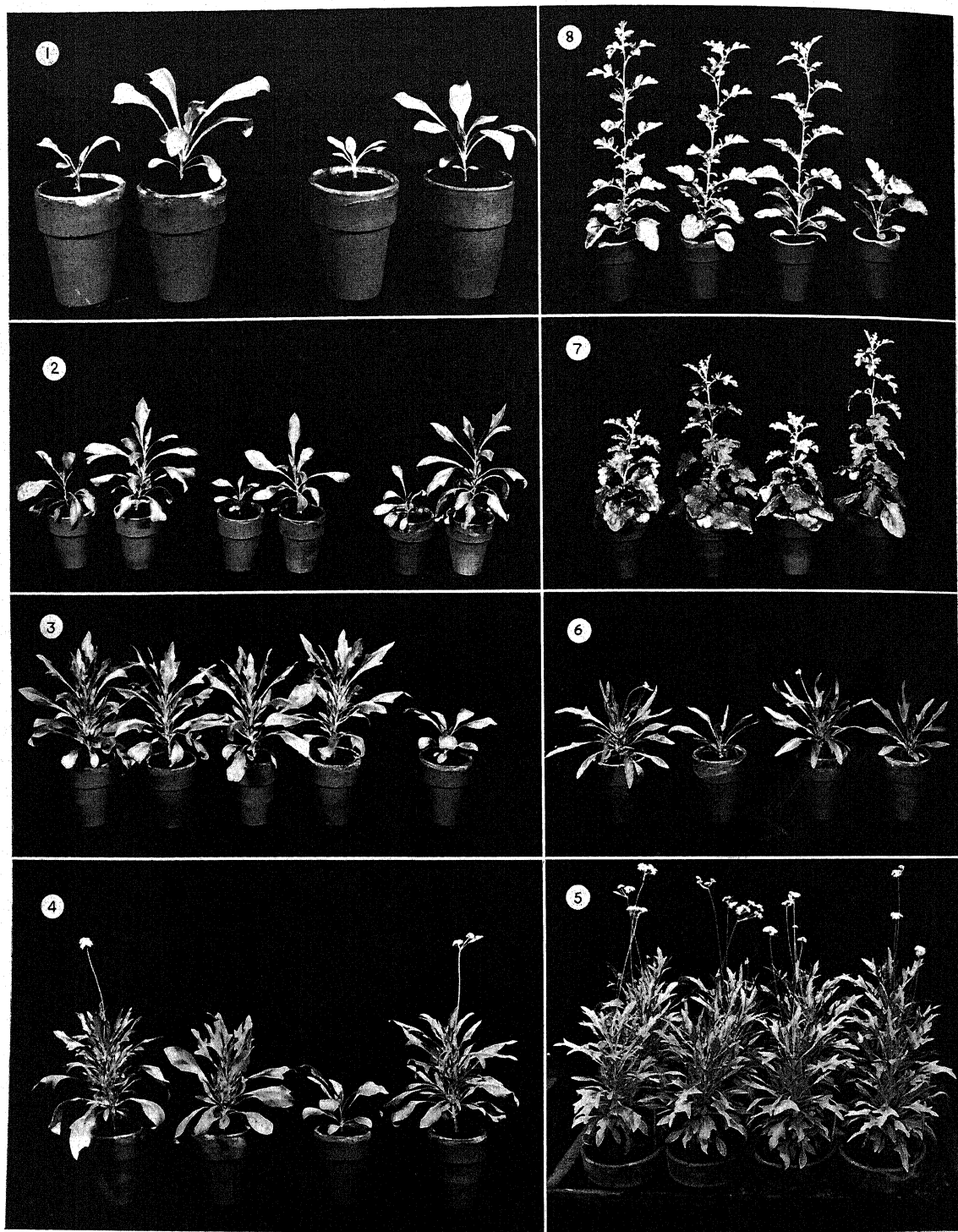


Fig. 1-8.—A comparison of deviating maternal plants and strictly maternal plants of guayule and mariola of the same age and progeny. All plants are in pots which measure about $2\frac{1}{2}$ inches in diameter at the top except those in figure 5. The latter are in pots 4 inches in diameter.—Fig. 1. Two pairs of apomictic guayule plants. The pair at the left were offspring of plant 593-V and were 53 days old. The pair at the right were 45-day old offspring of plant 593-I. The smaller plants are deviating maternals in each case.—Fig. 2. Three pairs of apomictic guayule plants. The two pairs at the left were (l. to r.) 90- and 78-day old offspring of plant 42440-I. The smaller plant is the deviating maternal in each pair.—Fig. 3. Five 83-day old apomictic offspring of guayule plant 593-I. The four plants at the left were strictly maternal, the one plant at the

TABLE 1. *Classification of the apomictically produced guayule plants obtained in eleven F₁ progenies resulting from interspecific pollinations.*

Plant number of female parent	Progenies	Total plants	Strictly maternal	Deviating maternal	Deviating maternal	Reduced maternal
	number	number	number	number	per cent	number
<i>2n</i> = +36 chromosomes (haploids)						
42354-I	1	13	12	1	7.6	0
6879-17	1	70	63	7	10.0	0
44-1277-1	1	24	20	4	16.6	0
Total	3	107	95	12	11.2	0
<i>2n</i> = ±72 chromosomes*						
42268-IV	1	38	38	0	0	0
416-II	1	65	63	2	3.0	0
593-V	3	190	181	9	4.7	0
42440-I	1	68	62	5	7.4	1
593-VIII	1	51	47	4	7.8	0
593-I	1	82	72	10	12.2	0
Total	8	494	463	30	6.0	1

* Each plant of the six included under this heading possessed 72 chromosomes of about the same size plus 2 chromosomes of very much smaller size.

table 1. These data show that the plants of only one progeny of the eleven were completely uniform. In all the others, there were some plants which deviated from the uniform majority. In some cases the deviating plants showed markedly slower growth when compared to sister plants of the same age, while in others the differences were not as great (fig. 1-8). Since the environment was uniform for all the plants involved, it seems justifiable to assume that genetic differences were responsible for the variation present within the same age-group of a given progeny. Furthermore, since a great majority of the plants of each progeny were strictly maternal, it seems clear that some type of genetic segregation occurred in the mother plant of each progeny either prior to or during the course of the formation of the seeds which gave rise to the deviating maternal plants.

It was known from previous studies (Bergner, 1944; Stebbins and Kodani, 1944; Powers and Rollins, 1945) that "haploids" from 72-chromosome strains occurred in guayule. Powers and Rollins (1945) have shown that this type of plant arises by reduced pseudogamy. Preliminary tests showed that a number of "haploid" plants produced viable seeds, and that they were largely formed apomictically. It was reasoned that if the variation found to occur among the apomicts of a given age-group of a progeny of 72-chromosome plants were assuredly attributable to genetic rather than environmental factors,

then "haploids" derived from 72-chromosome plants should show similar results. The data of table 1 show that this was the case. Plant 44-1277-1 was an apomictic daughter of plant 6879-17, and a chi-square homogeneity test shows that there is no significant difference between these two plants with respect to the number of deviating maternal plants produced. This was to be expected if plant 44-1277-1 arose through nonsegregating apomixis, as was presumed to be the case on the basis of a comparison of this plant with other members of the same progeny.

Similar variations to those in guayule were found to occur among the strictly apomictic portions of two F₁ progenies of mariola. Due to an oversight, quantitative data showing the frequency of occurrence of the deviating maternal plants were not taken. However, the percentage of deviating maternals was known to be less than five per cent of the total number of apomicts in each progeny. Figures 7 and 8 show strictly maternal and deviating maternal plants of mariola.

The whole evidence thus far considered leads to the conclusion that the variation found within the apomictically produced portions of F₁ progenies of both guayule and mariola is of genetic origin.⁴ A per-

⁴ Progeny tests of the deviating maternals were desired, but were impossible to obtain due to the unavailability of the necessary assistance. It should be borne in mind that progeny testing in this material is complicated by the presence of facultative apomixis and the present lack

right was a deviating maternal.—Fig. 4. Two pairs of apomictic guayule plants. The pair at the left were offspring of plant 593-V. The right hand plant of the pair shows only slight deviations from the strictly maternal plant at the left. The pair at the right were offspring of 593-I. The left hand plant of this pair shows marked deviations from its strictly maternal mate at the right. All four plants were 92 days old.—Fig. 5. Twelve strictly maternal guayule plants. They were the offspring of plant 416-II and were about 4 months old.—Fig. 6. Two pairs of apomictic guayule plants with about 36 chromosomes. The pair at the left were offspring of plant 6879. The pair at the right were offspring of plant 44-1277-1. The smaller plants were the deviating maternals in each case.—Fig. 7. Four apomictic mariola plants 109 days old. All were offspring of plant 43684-III. The smaller plants were deviating maternals.—Fig. 8. Four apomictic mariola plants 124 days old. All were offspring of plant 4275-VI. The three plants at the left were strictly maternal, the one at the right was a deviating maternal.

tinant question is, what is the source of this genetic variation among plants which upon *a priori* grounds would be expected to be uniform. Since only maternal chromosomal material is involved in the production of the deviating maternal plants, the assumption that some type of genetic (or cytological) change occurred during the production of this type of plant is inevitable. Whether this change occurred in the somatic tissue (Jones, 1941) of the mother plant prior to the formation of the reproductive structures, in the generative tissue, or subsequently, during the formation of the female gametophyte, cannot be determined from the data available. No cyto-embryological study of guayule and mariola has been made or is contemplated for the purpose of attempting to establish what mechanism in the parent plants provided for the genetic segregation evident. Nevertheless, some evidence as to whether the loss or addition of whole chromosomes was involved, was obtained by carefully determining the chromosome number of some of the deviating maternal plants in comparison with the chromosome number of the strictly maternal plants of the same progeny.

Plant no. 42440-I possessed $2n=72$ chromosomes of about the same size plus two chromosomes of definitely smaller size. An examination of five strictly maternal F_1 plants from plant 42440-I gave the same results. All five deviating maternals of this same progeny also possessed 72 chromosomes of about equal size and two smaller chromosomes. In working with chromosome numbers as large as these, there are chances for error, but the slides were repeatedly studied and many preparations were made in an attempt to minimize the errors in counting. Chromosome counts were made on at least one or two deviating maternal plants of the other progenies where they were present. In each case the chromosome number was alike in the deviating maternal plant and the strictly maternal plants of the same progeny. Even though a chromosome number analysis was not made for all the deviating maternal plants obtained, the sample so analyzed indicated that change in chromosome number was not an important factor in producing the genetic differences obtained.

The pollen size and trichome size of all deviating maternal plants of all progenies of guayule included in this study were determined and compared with strictly maternal plants of the same progeny in each case. The size-range of the pollen and of the trichomes was not widely different from that of the strictly maternal material. If the deviating maternal plants had possessed a chromosome number differing as much as 18 chromosomes, size differences in both pollen and trichomes would have been easily detected (Bergner, 1944; Rollins, 1944, 1945; Powers, 1945). Thus it is certain that even in those deviating maternal plants where chromosome counts were not made, the chromosome number did not differ to any large degree from that of the strictly maternal plants of the same progeny.

of knowledge as to how apomixis is inherited in guayule and mariola.

Apomixis in guayule has been shown by Esau (1944) to be of the type of generative apospory. That the application of pollen is essential for the production of viable seed in guayule and mariola has been shown (Powers and Rollins, 1945; Rollins, 1945). Therefore, apomixis in these species is pseudogamous. That the deviating maternal plants among the progenies of at least three plants given in table 1 were also obtained by pseudogamy, is shown by unpublished data on plant 593-V, 42268-IV and 42440-I. Where emasculation and nonpollination were practised on these plants, no viable seeds were produced. These data are to be presented in another publication.

Discussion.—Previously a single plant comparable to the deviating maternal plants of the present paper was reported by Powers and Rollins (1945), and it was there suggested that it probably arose by "pseudogamous diplospory." The likelihood of this particular plant having arisen as a result of contamination seemed extremely remote, since pollen control was demonstrated to be entirely effective. Furthermore, even if a small amount of contamination occurred, the chances of a few pollen grains being effective in normal fertilization in a highly apomictic mother plant makes the possibility of the daughter plant in question being one which arose by normal sexuality even more unlikely. The possibility that somatic segregation took place affecting the tissues which gave rise to the functional gametophyte was not discussed.

Since the present study was completed, Powers (1945) has interpreted a portion of his data as indicating that diplospory occurs as a method of reproduction in the offspring of a certain unusual "intermediate type" plant of guayule from Texas. His results clearly show that genetic segregation occurred among the normal plants produced in first, second and third generation progenies. However, a definitive separation into two groups of the normal plants which showed evidence of having arisen by some method of reproduction allowing segregation to occur, depending upon whether they arose by sexual reproduction or by diplospory, was not made. The clear demonstration that diplospory (or somatic segregation) occurred in this material was dependent upon an effective separation of the plants into these two categories. It is doubtful whether this separation could have been made without the benefit of wide crosses and rigorous pollen control, or the establishment of the actual extent of heterozygosity present in the parental types for comparative purposes. In spite of the possibility that sexuality may have accounted for all of the instances where genetic segregation was shown to have occurred, the probability that this was not the case is indicated by the results presented above.

Our own data show that genetic segregation occurred in a number of guayule and mariola plants prior to, or during the genesis of a low percentage of their apomictically produced offspring. This segregation could have taken place as a result of genetic

changes in the somatic cells, ultimately giving rise to the female gametophyte; or it may have occurred during the formation of the gametophyte as a result of atypical mitotic nuclear divisions. Jones (1941) has reviewed the literature of somatic segregation and listed some of the ways in which this type of genetic segregation may occur. Genetic changes occurring within the somatic body of the same plant give rise to chimeras (Neilson-Jones, 1937). These may be detected easily where superficial portions of the plant are visibly affected, but undoubtedly visible differences are not present in by far the greatest proportion of chimeras occurring in plants. That chimeras may be an important source of genetic variation has been shown for citrus by Frost (1926) and in Webber and Batchelder (1943). Visible chimeras are not rare in guayule and have been repeatedly observed. In some of these, observable differences are present in parts of leaves or stems, while in others, whole buds or branches differ from the rest of the plant. However, no chimeras were seen in the guayule and mariola material used in the present study. Nothing is known concerning chimeras in guayule and mariola where differences between tissues or organs of the same plant were not apparent. Somatic segregation resulting in chimeras where whole flowers, flower-heads or a portion or all of a flowering branch deviated from the rest of the plant must be considered as a possible explanation for the results obtained in the breeding experiments reported above.

A more plausible explanation may be obtained by assuming that imperfect mitoses, having some meiotic characteristics, occurred during the formation of the female gametophytes. The work of Gustafsson (1935, 1939), Gentscheff and Gustafsson (1940) and Fagerlind (1940) has emphasized the interrelationship of mitosis and meiosis particularly in the generative cells of apomictic plants. It has been shown that nuclear divisions occur in which the chromosomes are not reduced in number, but some or many of the characteristics of meiosis may be seen. Such divisions in the megaspore mother cell may or may not be followed by the production of diplospores. Transitional types of nuclear divisions in the female organs of a number of apomictic plants have been found (Gustafsson, l.c., Stebbins, 1941). The combination of nonreduction of chromosomes with the synapsis of at least some of the chromosomes in a single nuclear division would provide the conditions necessary for a logical explanation of our results. This would allow for both genetic segregation and nonreduction to occur in the formation of a given female gametophyte. Thus, the differential characteristics of the deviating maternal plants described in our experiments might easily have arisen as a result of the occurrence of synapsis followed by genic segregation in a few to several of the chromosomes.

Undoubtedly pseudogamy is a later phylogenetic development than sexuality, and pseudogamous plants probably arose from sexual plants. Aside from the physiological changes involved, the mor-

phological and cytological changes which took place in converting a sexual type to an unreduced pseudogamous type must have been gradual, as postulated by Powers (1945). The series of changes with which we are most concerned involved the displacement of meiotic nuclear divisions with mitotic divisions in the cells giving rise to the female gametophyte. It is unlikely that these changes occurred all at once. Rather, it is more probable that the change from meiosis took place gradually. The fact that unreduced pseudogamy in guayule and mariola as well as in other plants is facultative, supports this idea. Furthermore, it is usually assumed that the cellular environment of the megaspore mother cell, in sexually reproducing plants, is largely responsible for its development from a potential somatic cell into a generative cell whose nuclear contents divide by meiotic divisions. The change to mitotic divisions as in generative apospory reflects a change in this environmental influence. Since the environment is complex, and major changes would involve a whole pattern, it is doubtful whether this would have taken place climactically. Thus, intermediate stages of nuclear division having some of the characteristics of mitosis and some of meiosis might reasonably be expected where the environmental control was not complete for one type of nuclear division or the other.

If it is conceded that nuclear divisions intermediate between meiosis and mitosis occur in the development of functional female gametophytes in plants, then it seems probable that this type of gametophyte development also takes place in guayule and mariola. Furthermore, it is probable that diplospory is commonly involved in the production of the deviating maternal plants obtained in the experiments described above. Under these circumstances, the nuclear divisions preceding megaspore formation are not strictly apomeiotic (Stebbins, 1941).

SUMMARY

The apomictically produced portions of eleven F_1 progenies of guayule and two F_1 progenies of mariola, all resulting from interspecific pollinations, were grown for four months under uniform environmental conditions. Comparisons of plants of exactly the same age within these progenies showed that most of them were so nearly alike that they could not be distinguished from each other. However, in all but a single progeny, a small proportion of the plants deviated from the uniform majority. Since the environmental factors influencing growth were kept exceedingly uniform for all of the plants grown, it was concluded that the phenotypically different plants arose because of some type of genetic segregation in the mother plant in each instance.

Chromosome counts on a number of the deviating maternal plants showed that they possessed the same chromosome number as their parent. One haploid plant was found. Since normal meiosis could not have been involved in the production of the female gametophytes ultimately giving rise to the unreduced deviating maternal plants, the genetic segregation

which occurred must have taken place in some other way. Somatic segregation was suggested as a possibility, but it was considered that nuclear divisions with some features of meiosis and some of mitosis preceding gametophyte formation offered a more plausible explanation. If the latter were true, then

it was assumed that the deviating maternal plants arose most frequently by pseudogamous diplospory or semiapospory.

NATURAL HISTORY MUSEUM,
STANFORD UNIVERSITY, CALIFORNIA

LITERATURE CITED

- BERGNER, A. D. 1944. Guayule with low chromosome numbers. *Science* 99:224-225.
- ESAU, KATHERINE. 1944. Apomixis in guayule. *Proc. National Acad. Sci.* 30:352-355.
- FAGERLIND, F. 1940. Zytologie und Gametophytenbildung in der Gattung *Wikstroemia*. *Hereditas* 26: 23-50.
- FROST, H. B. 1926. Polyembryony, heterozygosis, and chimeras in *Citrus*. *Hilgardia* 1:365-402.
- GENTCHEFF, G., AND A. GUSTAFSSON. 1940. The balance system of meiosis in *Hieracium*. *Hereditas* 26:209-249.
- GUSTAFSSON, A. 1935. Studies on the mechanism of parthenogenesis. *Hereditas* 21:1-112.
- . 1939. The interrelation of meiosis and mitosis. I. The mechanism of agamospermy. *Hereditas* 25: 289-321.
- HOAGLAND, D. R., AND D. I. ARNON. 1939. The water-culture method for growing plants without soil. *California Agric. Exper. Sta. Circ.* 347:1-39.
- JONES, D. F. 1941. Somatic segregation. *Bot. Rev.* 7:291-307.
- MEYER, J. R. 1945. Prefixing with paradichlorobenzene to facilitate chromosome study. Submitted to *Stain Technology* for publication.
- NEILSON-JONES, W. 1937. Chimeras: a summary and some special aspects. *Bot. Rev.* 3:545-562.
- POWERS, LeROY. 1945. Fertilization without reduction in guayule (*Parthenium argentatum* Gray) and a hypothesis as to the evolution of apomixis and polyploidy. *Genetics* 30:323-346.
- , AND E. J. GARDNER. 1945. Frequency of aborted pollen grains and microcytes in guayule, *Parthenium argentatum* Gray. *Jour. Amer. Soc. Agron.* 37:184-193.
- , AND R. C. ROLLINS. 1945. Reproduction and pollination studies on guayule, *Parthenium argentatum* Gray and *P. incanum* H. B. K. *Jour. Amer. Soc. Agron.* 37:96-112.
- ROLLINS, R. C. 1944. Evidence for natural hybridity between guayule (*Parthenium argentatum*) and mariola (*Parthenium incanum*). *Amer. Jour. Bot.* 31:93-99.
- . 1945. Interspecific hybridization in *Parthenium* I. Crosses between guayule (*P. argentatum*) and mariola (*P. incanum*). *Amer. Jour. Bot.* 32:395-404.
- STEBBINS, G. L., JR. 1941. Apomixis in the angiosperms. *Bot. Rev.* 7:507-542.
- , AND MASUO KODANI. 1944. Chromosomal variation in guayule and mariola. *Jour. Hered.* 35:162-172.
- WEBBER, H. J., AND L. D. BATCHELOR. 1943. The citrus industry, Vol. 1. University of California Press. Berkeley and Los Angeles.

A CYTOGENETIC STUDY OF POLYEMBRYONY IN ASPARAGUS OFFICINALIS L.¹

Thomas E. Randall and Charles M. Rick

IN a review of polyembryony, Braun (1860) mentions the observation by Mettinius of multiple seedlings in garden *Asparagus*, *A. officinalis* L. The appearance of several twin seedlings in cultures of *Asparagus* at the California Agricultural Experiment Station in 1939 and 1940 stimulated the present study. This species, having an abundance of multiple seedlings, is an attractive subject for an investigation of the nature and origin of polyembryony.

Since the literature of polyembryony has been reviewed and the applications of this phenomenon in cytogenetic research and plant breeding have been discussed recently by Webber (1940), these aspects need not be treated here except as they apply to polyembryony in *Asparagus*.

Two techniques serve as avenues of approach in ascertaining the origin and development of multiple seedlings arising from a single seed. One is a study of the embryological development, chiefly by means of microtome sectioned material. The other is a study of the cytogenetic characters of plants that originate

as multiple seedlings; in this way, the mechanics of fertilization and subsequent development may be deduced. The developmental method, when properly interpreted, provides a means of absolute determination. Its chief disadvantage is that only a few ovules can be secured at different stages of development, because of the commonly very low frequency of polyembryony. The use of this method is well exemplified in the recent work of Cooper (1943), establishing the origin of haploid-diploid twin embryos in *Lilium* and *Nicotiana*. The genetic method offers a way to discriminate accurately between certain types of development; for example, a monozygotic origin can be distinguished from a dizygotic origin by resemblances or differences in genetic characters. The chief limitation is that this method cannot distinguish certain types of polyembryonic development. For example, it cannot indicate which specific nuclei function in cases where only a single embryo sac is involved. In the present survey the genetic approach is chiefly used, although certain developmental evi-

¹ Received for publication June 5, 1945.

dence is also provided by a study of gross morphological details during germination.

To ascertain the range of frequencies of polyembryony, seed samples were tested from as many diverse sources as feasible. Of 36 lots of seed, five were obtained from controlled pollinations; five represented different horticultural varieties, in each of which seed was massed from several plants; the remaining 26 were obtained from various open-pollinated pistillate plants, mostly derived from the variety Mary Washington. These pistillate plants were subject to considerable hybridization with many staminate plants of unrelated lines, which might have masked differences between pistillate plants; nevertheless, the seed lots thus obtained were genetically different as witnessed by the differences in many seedling characters. Mr. G. C. Hanna of the California Experiment Station kindly furnished most of the seeds used in this work.

The seeds were planted in flats of sand by means of a vacuum-operated seed-counting plate in order to space uniformly, thereby facilitating the subsequent detection of multiple seedlings. Five hundred or more seeds of each lot were sown. Germination was satisfactory, usually amounting to 80 per cent or higher. Frequencies were ascertained when germination had been completed—that is, from four to six weeks after sowing.

Chromosome numbers were determined in microtome sections of root tips. Root tips collected when seedlings were removed from the sand were generally satisfactory for this purpose. For confirmatory counts, however, root tips were collected from the seedlings growing in soil to which they had been transplanted after removal from the sand. The determination of chromosome number was often repeated independently in several tips where the number was twenty—the normal diploid count—and always repeated where a heteroploid number was observed. The very high percentage of agreements between such repeated observations attests the reliability of single counts. Root tops were fixed in Navaschin's fluid and stained with iodine-crystal violet according to Newton's method.

Frequency of polyembryony.—In all, 405 multiple seedlings were obtained in the germination tests. Table 1 shows their frequencies in the 36 seed lots tested. The mean frequency of all seed lots is 0.95 per cent. This average of nearly one per hundred is considerably higher than the usual frequency of polyembryony observed in angiosperms, according to Webber's review (1940).

The lowest and highest frequencies observed were, respectively, 0.13 per cent in a seed lot that was the product of a controlled cross, and 3.54 per cent observed in a massed seed lot of the variety Argenteuil. Statistically the difference between these two figures and between many other paired frequencies is highly significant, indicating an influence of the genotype upon the frequency of polyembryony. Thus, selection might increase the production of multiple seedlings in *Asparagus*. Kappert (1933) increased the

TABLE 1. Frequency distributions of the percentages of different types of multiple seedlings in 36 tested seed lots.

Percentage class mean ^a	Separate multiple seedlings	Conjoined multiple seedlings ^b	Total multiple seedlings
0.20	19	23	9
0.60	10	9	12
1.00	3	2	6
1.40	1	..	4
1.80	2
2.20	1	2	1
2.60	3
3.00
3.40	1
Total	36	36	36

^a Class interval is 0.40 per cent.

^b Triplets and quadruplets conjoined in any manner are classed as conjoined multiple seedlings.

yield of multiple seedlings in *Linum usitatissimum* from 0.01–0.04 to 8–9 per cent by selection in four generations.

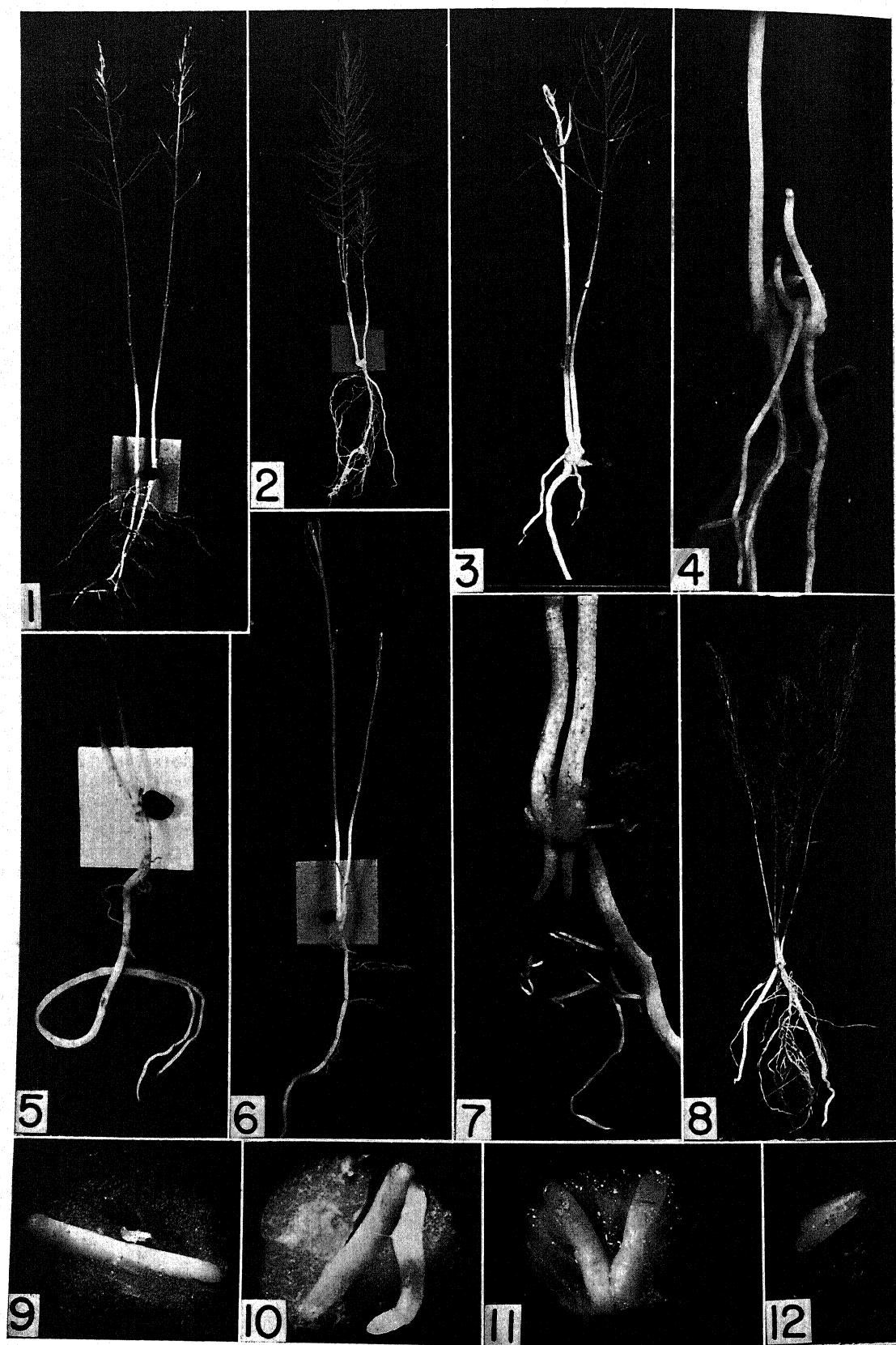
The conditions of germination may affect the frequency of multiple seedlings observed. Thus, when the total percentage germination was reduced by adverse conditions—for example, abnormally high temperature—the percentage of polyembryonic seedlings decreased markedly below the percentage of the same lot germinated under more favorable conditions. Probably not all of the multiple embryos present in dormant seed develop into seedlings, even under highly favorable circumstances. Certain embryos (a typical example appears in fig. 4) are comparatively very small; it is unlikely that these always survive in competition with their larger mates. A similar effect of environment was observed by Kappert (1933).

Description of multiple seedlings.—Two or more seedlings emerging from a single seed are defined as a *multiple seedling*; the non-multiple or normal type, in which the sole product of germination of a seed is a seedling having a single shoot and single primary root, is referred to as a *single seedling*.

The multiple seedlings encountered in this survey can be conveniently described according to three aspects: morphology, number of members, and chromosome number.

A striking morphological feature is the frequent attachment existing between members of a multiple seedling. Multiple seedlings may be readily classified, therefore, into *separate* and *conjoined* types.

The *separate* type is defined as the multiple seedling between whose members no direct morphological attachment exists. Typical examples are illustrated in figures 1–4. Each seedling draws upon endosperm reserves by means of its own cotyledon and at the time of germination may be removed individually from the seed and grown to maturity without difficulty. Each exists as a separate embryo in the seed (fig. 10, 11). The percentages of separate



type multiple seedlings in the 36 seed lots are given in table 1.

TABLE 2. Frequencies of the different types of multiple seedlings.

Type	Number	Percentage
Separate twin seedlings	222	54.8
Conjoined twin seedlings.....	171	42.2
Triplet seedlings.....	11	2.7
Quadruplet seedlings.....	1	0.3

* Members are conjoined to varying degrees in six of the 12 triplets and quadruplets.

For convenience the designation "conjoined" is given to seedlings whose members are morphologically attached to each other. Of the 405 multiple seedlings obtained, 171 (42.2 per cent) were conjoined, 222 (54.8 per cent) were separate, and 12 (3.0 per cent) consisted of three or more members (table 2). The degree of attachment varies from a slight bond of tissue at the hypocotyl to complete union save a bifurcated shoot or root. Often the conjoined parts are distinctly fasciated, as illustrated in figure 5. Attachment always involves the hypocotyl region and usually part or all of the primary root. The multiple seedling possessing a double shoot and single primary root (fig. 6) is the type most frequently encountered.

Asparagus seedlings have been observed to develop double shoots or double roots as a result of injury to the growing point during sprouting of the seed. This type might be classified with conjoined twins since, as will be demonstrated in a following section, both are mainly derived by incomplete cleavage of a single initial embryo. The type resulting from injury differs from the conjoined type, however, in the course of subsequent development and in the higher frequency of heteroploid chromosome numbers in the latter group.

The intrinsically double nature of the conjoined type can be demonstrated four to six weeks after germination, when the majority develop separate growing points. Each of the two crowns produces its own set of roots and shoots, the two remaining conjoined only at the original locus of attachment. Figure 7 illustrates the emergence of roots, each below one of the two separate shoots originally conjoined by a single root shown at the right. Figure 8

shows a later stage in the independent growth of a conjoined twin seedling, which earlier had resembled the twin seedling in figure 6.

A sample of 34 conjoined twin seedlings was inspected six weeks after germination—that is, about ten weeks after sowing. Of these, 26 had already developed separate systems of roots and shoots; they could be separated readily and grown as individual plants. By this time the bond of tissue connecting members had disintegrated in many of these twins. Within six months the remainder could be divided.

This test of the independent condition of multiple seedlings of the conjoined type would not be valid if seedlings of the single type could be similarly divided. However, one cannot split seedlings of the single type into viable divisions at comparable stages of growth. Thus, after four months of growth, only one seedling could be successfully divided in a sample of 28 single seedlings derived from a plant that produced a high percentage of conjoined twins.

Braun (1860) describes and illustrates instances of similar attachment in the polyembryony of *Alchornea* (*Coelebogyne*) *ilicifolia*. The same phenomenon was observed by Kappert (1933) in *Linum* and by Webber (1938) in *Gossypium*.

In respect to the number of members, the multiple seedlings were nearly all twins; only three per cent consisted of three or more members. Of the latter, eleven were triplet seedlings, one a quadruplet. In these higher multiples the relation among members varies from complete separation of all members to union of all. One or more members were usually very weak, and many seedlings failed to survive, so that the survey of chromosome numbers for this group is very incomplete.

Tables 3 and 4 summarize the distribution of chromosome numbers in twin seedlings of the separate and conjoined type. Chromosome counts were made in all but 13 per cent of the plants derived from twin seedlings. This proportion represented plants that failed to survive because of chlorophyll deficiency, of otherwise weak genotypic constitution, or of disease. The undetermined member was smaller in the majority of the twin seedlings whose other member was determined.

Typical of observations on polyembryony, the diploid number, 20, was the most frequent one observed. The heteroploid plants obtained exhibited the following chromosome numbers: 30 (triploid), 21 (trisomic), 10 (haploid), and 40 (tetraploid),

Fig. 1-12.—Fig. 1-8. Various types of multiple seedlings photographed at several stages of growth following germination.—Fig. 1. Typical separate type twin seedling in which both members are diploid. $\times \frac{1}{2}$.—Fig. 2. Separate twin seedling in which the left member is diploid, the right, haploid. $\times \frac{1}{2}$.—Fig. 3. Separate pair in which left member is a recessive chlorophyll-deficient seedling, the right, a normal green seedling. $\times \frac{1}{2}$.—Fig. 4. Separate type triplet. Shoot of the leftmost member is approximately twenty times as long as that of the rightmost member. Chromosome numbers undetermined. $\times 2$.—Fig. 5. Conjoined type diploid twin seedling showing fasciation of the greater part of the existing primary root. $\times 1$.—Fig. 6. Conjoined diploid twin seedling of the most frequent type having a primary root single throughout its length. $\times \frac{1}{2}$.—Fig. 7. Initiation of roots from each of two independent crowns of a conjoined twin seedling similar to that in figure 6. $\times 4$.—Fig. 8. Later stage in the independent growth of two crowns originating as in figures 6 and 7. Note the nearly identical growth rate of the two seedlings. $\times \frac{1}{2}$.—Fig. 9-12. Seeds dissected in order to expose embryos. $\times 10$.—Fig. 9. Typical seed with single embryo.—Fig. 10 and 11. Twin embryos with no morphological connection between members.—Fig. 12. Twin embryo conjoined in a fasciated manner throughout its length.

TABLE 3. *Distribution of chromosome numbers among the larger and smaller members of separate twin seedlings.*

Number of twins whose larger member has the chromosome number	Number of twins whose smaller member has the chromosome number					Undeter- mined
	10	20	21	30	40	
10	1	2
20	3	150	2	5	1	27
21	..	5
28	..	1
30	..	5	..	1
Undetermined	..	8	1	10

listed in order of observed abundance. A plant with 28 chromosomes also appeared in a twin seedling. The relatively high frequency of trisomics was unexpected according to previous observations on polyembryony (cf. Webber, 1940). A diploid member was present in every pair of separate twins except in two cases in which the two members were identical for a deviating number.

Table 3 indicates the chromosome number of the larger and the smaller members of separate twin pairs. Relative size was determined at the time of germination. As in figure 2, the haploid member of the haploid-diploid pairs was always much smaller than its diploid mate. In other associations of unlike chromosome numbers, the heteroploid member displayed greater vigor as often as the diploid member. Twin seedlings whose members were nearly identical in size, and were very similar to each other in other morphological respects, very often possessed the same chromosome number. Otherwise, and except for the haploid-diploid twins just mentioned, the degree of difference in size seldom gave any clue as to chromosome number or the origin of polyembryony—an observation contrary to the findings of Müntzing (1937).

TABLE 4. *Summary of chromosome numbers of twin seedlings.*

Chromo- some numbers	Plants derived from separate twin seedlings		Conjoined twin seedlings	
	Number	Per cent ^a	Number	Per cent ^a
10	7	1.8	3	2.1
20	357	92.5	138	95.2
21	8	2.1	3	2.1
28	1	0.3
30	12	3.1
40	1	0.3	1	0.7
Undetermined	58	..	26	..
Total	444	..	171	..

^a Per cent of those whose chromosome number was determined.

The distribution of chromosome numbers in the conjoined twin seedlings closely resembles that of the separate type (table 4). A noteworthy excep-

tion is the complete absence of triploids in the conjoined group.

Although only ten haploids survived to the seedling stage in this experiment, many more may have perished before their chromosomes were counted. Many lethal genes and weak genotypes exist in this species, and even the best available strains display considerable variation. This highly heterozygous condition is expected because propagation solely by cross-pollination is ensured by the dioecious condition and because uniformity has not been sought consistently in former breeding work and seed production. Presumably only the haploids that are fortunate in possessing non-lethals and an otherwise favorable genotype survive germination. Since it is usually paired with a diploid embryo, whose weak genes would mostly be masked by stronger alleles, the haploid embryo is subject to extreme competition. Furthermore, certain evidence indicates that haploidy *per se* results in a less viable sporophyte. Thus, in tomatoes, haploid clones are considerably weaker than the diploid clones of identical genotype derived by chromosome doubling from the haploids (Rick, unpublished). It seems significant in this connection that the haploid seedling is always much smaller than the accompanying diploid (table 3, fig. 2).

The production of homozygous diploids by doubling the chromosome number of haploid sporophytes has often been advocated as a quick method of obtaining pure lines. In cross-pollinated crops such as *Asparagus*, this method would offer the additional advantage of selecting the most vigorous genotypes, the weaker ones being eliminated by the severe selection in polyembryonic development and in later growth of the seedling. The vigorous genotypes thus selected might prove valuable horticultural material *per se*, or they might serve as useful inbred lines for the production of F₁ hybrid seed. To double the chromosome number of the *Asparagus* haploids would serve no immediate purpose, since, by virtue of the sex-determining mechanism of this species, all parthenogenetic haploids are pistillate and thus incapable of sexual propagation by themselves. If, however, homozygous staminate lines could be found, the opportunity of utilizing hybrid vigor would be realized. A method for obtaining such lines exists

TABLE 5. *Analysis of distribution of sex in twin seedlings of the separate type.*

	Combinations of sexes			Total	P
	♀♀	♂♂	♀♂		
Number of pairs observed.....	30	23	26	79	...
Number of pairs expected (random association)	23.4	16.4	39.2	79	...
χ^2 (1 d.f.)	1.86	2.66	4.44	8.96	<0.01
Number of pairs expected (one third monozygotic)	29.9	22.9	26.1	79	...

in the utilization of perfect flowers produced tetragametically by certain lines (Rick and Hanna, 1943). Since the seeds produced by these flowers probably arise from self-pollination, propagation for several generations by selection of these seeds would produce inbred staminate lines. This selection should also lead to rapid fixation of the male-determining factor. The combination of such inbred staminate lines with homozygous pistillate lines derived from haploids would therefore offer, in addition to hybrid vigor, the advantage of producing hybrid progeny consisting solely of staminate plants, which generally outyield pistillate plants.

Development of the separate type.—Cytogenetic studies were employed to reveal certain aspects of the origin of this type of polyembryony. The most conclusive evidence is provided by two Mendelian characters segregating in this material. Sex in this species, as in most dioecious angiosperms, segregates in a 1:1 ratio determined by a single factor (or chromosome) pair, the male sex being heterogametic (Rick and Hanna, 1943).

Sex of both members was determined in 79 twin seedlings of the separate type. All plants were diploid. The distribution of sexes observed is presented in table 5. Although staminate plants are deficient in this total, the deviation is too small to be significant.

A chi-square test is applied to determine whether the excess of twins identical for sex is significant. If the expected number of pairs were calculated for this test by assuming random association of equal numbers of each sex, the measure of error would include the deviation from normal sex ratio in addition to the deviation from the expected number of identical twins. The expected number is therefore calculated by assuming random association of the observed numbers of each sex. The same procedure is followed in calculating the expected values in table 6. If a represents the observed number of ♀ ♀ twins, b , the number of ♂ ♂ twins, c , the number of ♀ ♂ twins, and a' , b' , and c' , the corresponding expected values, this assumption may be represented as follows:

$$a + \frac{1}{2}c = a' + \frac{1}{2}c' = 43, \text{ and} \\ b + \frac{1}{2}c = b' + \frac{1}{2}c' = 36.$$

These conditions constitute one linear restriction in addition to the other implied in the assumption that

$$a + b + c = a' + b' + c' = 79.$$

Since there are three classes, these two linear restrictions leave but one degree of freedom. The chi-square thus obtained indicates a highly significant excess of twins identical for sex.

The combination of a pistillate and a staminate member proves the dizygotic origin of 26 of the total. The significant excess of twins identical for sex probably reflects the presence of an appreciable number of monozygotic twins. Although the data do not reveal the exact number of monozygotic twins present, a rough estimate can be derived by fitting to the data distributions expected on various assumptions. A very close fit to the observed numbers is obtained if one third of the total twins are assumed to be monozygotic, the remainder, dizygotic (table 5).

If the twin seedlings of identical sex include twins of monozygotic origin—that is, twins identical in all respects—then the resemblance in seedling length between members of a pair should be greater in the group identical for sex than in the group differing in sex. The mean ratio of seedling length (smaller/larger member) for the former group is 0.70; for the latter group it is 0.64. Thus, despite the relatively great variation in seedling length, even within such groups as are later proved to be monozygotic, this expectation is realized.

The other genetic character, purple anthocyanin coloration of the stem base and bracts of the newly emerged shoot, segregates as if conditioned by a single dominant basic color factor. The determination of the degree of coloration, being vastly more complex, will not be considered here. Inasmuch as the seed was mostly derived from open-pollination, green seedlings appeared, not in the usual one half or one fourth, but in smaller proportions of the total seedlings in these lots. Furthermore, so few seedlings are involved, that statistical comparisons are not warranted. The significant data are presented in table 6. The dizygotic origin of twins of differing sexes is verified, for stem colors are associated at random in twins of this group. As expected, some twins differing in color are found in the group that are identical for sex, but fewer of the former exist than are expected if colors are distributed at random. If one third of all separate twins are monozygotic, one half of those identical for sex should be monozygotic. The numbers expected on the basis of one half monozygotic twins in the group identical for sex provide a closer approximation than the estimate based on random distribution. The best fit is obtained

TABLE 6. Analysis of distribution of stem color in twin seedlings of the separate type.

	Combinations of colors			Total twins
	Purple purple	Green green	Purple green	
♀♀ and ♂♂ twins				
Number of pairs observed.....	29	3	7	39
Number of pairs expected (random association)...	27.1	1.1	10.8	39
Number of pairs expected (one half monozygotic)...	29.8	3.8	5.4	39
Number of pairs expected (one third monozygotic)	28.9	2.9	7.2	39
♀♂ twins				
Number of pairs observed.....	16	0	3	19
Number of pairs expected (random association)...	16.1	0.1	2.8	19

when one third of this group (that is, one fifth of the total separate twins) is assumed to be monozygotic. Considering the distribution of both sex and stem color, roughly one fourth of the separate twins can be assumed to be monozygotic.

Twins including a green member and a chlorotic member as in figure 3 were also observed. The progenies segregating for this recessive chlorophyll deficiency were too small for their frequency of twin seedlings to be useful in indicating the origin of the separate type.

Another fact suggests the monozygotic origin of a portion of the separate type—namely, the appearance of an unexpectedly high proportion of multiple seedlings that are identical for a heteroploid chromosome number. For instance, one triplet was composed entirely of separate members, two of which showed a trisomic count, the third an uncertain count, being either diploid or trisomic. Two other twin seedlings were haploid-haploid and triploid-triploid. Furthermore, in two other pairs in which only the haploid member was counted, comparative morphology strongly indicated that the uncounted member was also haploid. These multiple seedlings with identical chromosome number appear twenty, seven, and three times as often as would be expected on a random distribution of chromosome numbers in the haploid, trisomic, and triploid groups respectively. Yet the appearance of these identical types could readily be explained by the cleavage of a single incipient zygote or embryo.

Kappert (1933), led by similar evidence, concludes that many diploid-diploid twin seedlings in *Linum* originate in the same manner. In *Linum*, however, the twins derived from heterozygous parents were nearly all identical in genotype, the few exceptions being evidently haploid-diploid combinations. Other examples of cleavage polyembryony are cited from the literature by Webber (1940).

In *Citrus* and certain other genera, identical multiple seedlings often are the products of nucellar polyembryony—that is, they develop directly from cells of the nucellus. The only evidence suggesting this origin for multiple seedlings in *Asparagus* is the fact that the number of ♀♀ twins exceeds that of ♂♂ twins, although the deviation from

the expected 1:1 ratio is not significant ($P = 0.34$). Nucellar polyembryony, moreover, could not explain the excess of ♂♂ twins in addition to ♀♀ twins above the expected numbers, nor the unexpectedly high frequency of twins identical for heteroploid chromosome numbers. If certain identical twins did originate in this manner, they would constitute a very small group in addition to those already proved to arise monozygotically and dizygotically. Thus, although nucellar polyembryony cannot be completely disproved, it is unlikely that it occurs in *Asparagus*.

The remaining three fourths of the separate twin seedlings—those whose sexes and stem colors are paired at random—are obviously of dizygotic origin. Since maleness in *Asparagus* behaves as a simple Mendelian dominant, for which normal staminate plants are heterozygous, all embryo sacs are identical in sex determination; but half of the male gametophytes carry the male determiner, the other half, the recessive factor. This group of twin seedlings, accordingly, must have arisen from ovules, each of which was penetrated by two pollen tubes.

The functioning of two pollen tubes in an example of polyembryony in *Triticum* has been discovered by Kasparyan (1938). Here members of a twin seedling having the somatic chromosome numbers 35 and 49 originated from the cross *T. vulgare* ($2n = 42$) \times *T. armeniacum* ($2n = 28$). The former seedling was evidently derived from the union of a reduced egg of *vulgare* and a reduced sperm of *armeniaceum*; the latter was the product of a reduced egg of *vulgare* and two reduced sperms or one nonreduced sperm of *armeniaceum*. Presumably another sperm fused with the polar nuclei to produce the endosperm. In this instance, therefore, a minimum of two pollen tubes would be required to contribute a total of four sperms. The functioning of multiple sperms has been convincingly established in the production of normal monoembryonic seeds of maize by Sprague (1932). By using appropriate crosses, Sprague discovered frequencies of this phenomenon at least as high as 25 per cent. Fertilization by the sperms of two pollen tubes might therefore be expected in normal embryogeny as well as in polyembryony of angiosperms.

The following information sheds light on the number of embryo sacs involved in this type of polyembryony. Of the twin seedlings whose mates differed in stem color, four appeared in the offspring of purple-stemmed pistillate plants. Since the seed in these instances was derived from open-pollination, the genetic identity of the staminate parents is unknown, although they had to be either homozygous for the recessive gene or heterozygous for the dominant color allele. Nevertheless, the pistillate parent must have been heterozygous because it was phenotypically purple and yielded a few green seedlings. In any event, it is necessary to assume different female gametes to explain the origin of the differently colored twin seedlings.

According to Flory (1932), development of the embryo sac in *Asparagus officinalis* follows the normal type. Thus, every nucleus of a mature embryo sac is identical genetically, but nuclei of different embryo sacs could differ if they were derived from different megaspores of a heterozygous plant. It follows, therefore, that twin embryo sacs gave rise to the differently colored twins. If by remote chance, however, twin seedlings should develop from exceptional embryo sacs that develop according to the *Scilla* type (particularly since in *Asparagus* the micropylar cell retains two megaspore nuclei) or any other pattern in which the nuclei of an embryo sac are derived from more than one megaspore, differently colored twins could develop from a single embryo sac. Though the facts suggest that multiple embryo sacs may lead to polyembryony in *Asparagus*, they are not sufficient to indicate what proportion of dizygotic twin seedlings might develop in this manner.

Though Flory does not report any twin embryo sacs, he did observe, in one instance, two megaspore mother cells within one ovule. Twin embryo sacs might also arise from two megaspores derived from the same mother cell. The following statements by Flory are of interest in this connection: "However, the cell wall that starts to form across the spindle of the secondary megasporocyte nearest the micropyle disappears instead of extending all the way across the cell and dividing it. Thus there results from the II division three megaspores . . . two of which are uninucleate, while the third one (the one nearest the micropyle) is binucleate." If the chalazal cell failed to develop, this pair of nuclei at the micropylar end might conceivably be stimulated to develop into two separate embryo sacs or a single embryo sac of a modified *Scilla* type.

In a recent paper, Pope (1943) describes two twin embryos in barley which were discovered in sectioned spikelets fixed ten and twelve days after pollination. On the grounds that the members of each pair were alike in having the diploid chromosome number, he assumes that both specimens are examples of cleavage polyembryony. The present study shows how unreliable a similarity of chromosome number alone can be in determining the origin of multiple seedlings; about three fourths of the

separate diploid-diploid twin seedlings in *Asparagus* do not arise from cleavage polyembryony.

The origin of the heteroploid types obtained remains largely conjectural. The haploid plants presumably develop by parthenogenesis from some haploid cell of the embryo sac. Cooper (1943) found that, in haploid-diploid twin embryos of *Lilium* and *Nicotiana* and perhaps in general, the haploid embryo develops parthenogenetically from a synergid and the diploid embryo, normally, from fertilization of the egg, all within the same embryo sac. The three haploids that have flowered in the present experiment are all pistillate. This condition is expected because a haploid plant developing parthenogenetically from a cell of the embryo sac would necessarily have the same genotype for sex as its mother.

Various origins have been attributed to triploids occurring in twin seedlings. Nothing in the present work would indicate how they arise in *Asparagus*. Trisomics, evidently the product of union of a haploid gamete with a gamete having an extra chromosome, are found as often as haploids and triploids. Nondisjunction of a chromosome in mitosis or meiosis might account for the unbalanced gamete. Conceivably, triploids themselves could also contribute such unbalanced gametes in the *Asparagus* fields. Triploids are equally as vigorous as diploids in twin seedlings and would be planted unwittingly with the diploids. Although the present study indicates a frequency of only one triploid per 2,000 (others might arise as seedlings of the single type), the triploids could still furnish a portion of the functioning gametes of the population.

Development of the conjoined type.—Obviously, the attachment between seedlings derived from the same seed can arise by one of two processes: either two incipient embryos become grafted to each other, or a single incipient embryo cleaves incompletely during development from the zygote to the seedling stage. The latter pattern of development is uniformly indicated by the evidence to be discussed.

If every twin seedling of the conjoined type arises from a single zygote, any pair of seedlings thereby developed should be identical in respect to their entire genotype. If the pair develop from two zygotes they would be expected to differ cytogenetically unless the two zygotes were produced by fusion of two identical female gametes with two identical male gametes. Studies on Mendelian inheritance in *Asparagus*, however, demonstrate that gametes unite at random and that fusion of identical gametes would occur very rarely. Cytogenetic evidence might therefore be expected to aid in solving this problem.

Chromosome number is a cytogenetic character that sheds light on this problem. As mentioned previously, certain conjoined twin seedlings were successfully separated. The independent halves were cultured in pots and their chromosome numbers ascertained. In 22 such twins whose chromosome numbers were satisfactorily determined, the count

was identical for each member of the pair. The members of a pair differed in chromosome number in 12.6 per cent of the 174 twin seedlings of *separate* type in which the number of both members was determined (table 3). Since data presented previously indicate that the majority of the *separate* type are dizygotic, at least three of these 22 conjoined pairs would be expected to differ in count if they originated from two initial embryos. This result would suggest that at least most of the attachment resulted from incomplete cleavage, although the numbers are too small to be significant. Also, if fusion occurs in development, it might conceivably occur more often between members of like chromosome number.

Seedlings of identical genetic constitution might be expected to grow at nearly the same rate. Thus members of a monozygotic pair would resemble each other to a greater extent than members of a dizygotic pair. In this respect, also, the evidence points to a monozygotic origin, for the similarity in seedling lengths is greater in the conjoined than in the *separate* twin seedlings. The latter, as demonstrated, are probably mostly dizygotic in origin. In both groups, the variation in ratios of seedling length is great; nevertheless, the mean ratio of smaller/larger member of a pair is 0.76 for the conjoined group, whereas the ratio is 0.66 for the *separate* group.

As in the *separate* type, the Mendelizing characters, sex and stem color, provide the best discrimination between a monozygotic and dizygotic origin. Thirty conjoined twin seedlings were separated and grown to the flowering stage. Without exception both members of each pair were identical in sex. The probability that a distribution of sex of this type would occur between individuals of dizygotic origin is 10^{-9} . Again, these results would be expected if fusion occurs only between members of like sex, but such preferential behavior seems very unlikely. Apparently, therefore, most if not all of the conjoined seedlings were monozygotic.

In respect to stem color, also, the members of every pair, including several green pairs, were identical. One may summarize the cytogenetic evidence, therefore, by stating that not one conjoined pair was found whose members differed for any of the three characters studied.

The universal attachment at the hypocotyl region and the tendency of the shoot and primary root to *separate* distally suggest that the cleavage might have occurred comparatively late in development. Though early embryonic development was not studied, favorable material was examined in order to follow development of the germinating seed.

The status of embryos was determined in dormant seed and compared with that in germinated seed of the same lot. Seed soaked in water at room temperature for four days was softened enough to be dissected with a pointed scalpel under a dissection microscope. In this species, the embryo, a slender, somewhat awl-shaped body, is completely in-

vested by the much larger body of horny endosperm. Figure 9 shows a typical seed with exposed single embryo. The greater portion of this embryo comprising the pointed end is the cotyledon, which always remains imbedded in the endosperm, acting as a haustorium responsible for the transfer of food reserves to the germinating seedling. On sprouting, the blunt end emerges from the seed and grows downward as the primary root. The shoot apex is situated a short distance posterior to the root tip, and shortly after sprouting it gives rise to a scale-like leaf about 2 mm. from the seed coat.

TABLE 7. Comparison of frequencies of *separate* and conjoined types of multiple embryos in dormant and germinated seed in nine seed lots.

	Multiple embryos in dormant seed		Multiple seedlings in germinated seed	
	Separate per cent	Conjoined per cent	Separate per cent	Conjoined per cent
	2.14	0.23	1.50	2.05
	0.42	0.42	0.79	0.53
	1.33	0.00	1.44	0.18
	1.41	0.00	0.74	1.10
	2.25	0.00	2.68	0.00
	0.69	0.00	0.19	0.76
	0.89	0.00	0.40	0.91
	0.65	0.00	0.23	0.15
	0.00	0.00	0.11	0.23
Mean	1.08	0.09	0.74	0.77
Total number of seeds examined	3,415		5,382	

In the dissected seeds no partially cleaved embryos were discovered, except three fasciated specimens in a total of forty twin embryos. Figure 12 illustrates the fasciated type; figures 10 and 11, the more frequent *separate* type. In the fasciated twin embryos, both members were connected throughout their length. According to the pattern of development just mentioned, the only attachment likely to remain in the seedlings from this type of embryo would be the fasciated cotyledon.

The frequencies of *separate* and conjoined embryos in dormant seed are compared with corresponding data for germinated seed in table 7. The frequency of *separate* and conjoined types in dormant seed is scarcely sufficient to account for the frequency of the two types in germinated seed. Fewer total multiple embryos appeared in dormant seed than in germinated seed in eight of the nine lots examined. Furthermore, the actual discrepancy is probably even greater than these data reveal: for reasons already mentioned, some seeds containing multiple embryos probably do not germinate or they yield only a single seedling. The frequency of both types of multiple embryos in dormant seed is high enough only to account for the *separate* multiple seedlings in germinated seed. Apparently, therefore, all or most of the *separate* multiple seedlings are derived from the *separate* multiple em-

bryos observed in dormant seed, and the conjoined types are developed from what appear to be single embryos with the possible exception of the fasciated embryos. Thus most of the outwardly detectable differentiation of a single embryo into a conjoined multiple seedling probably occurs during germination.

Many causes might be imagined for this cleavage in development. One possible clue is the fact that nearly five per cent of the conjoined seedlings were heteroploid (table 4). This proportion greatly exceeds that of seedlings of the single type in *Asparagus*. It suggests that this cleavage might involve only a certain specific type of seedling, as though the tendency to cleave were influenced by the genotype of the seedling itself. Possibly some element of the maternal environment serves as a common cause both for the deviating chromosome number and for the cleavage of the embryo.

Since the conjoined seedlings and the portion of the separate twins that are proved to be monozygotic both develop by cleavage of a single initial embryo, they are classified into two morphological types only for the sake of convenience and not to indicate their origin. Both types are end products of the same process, the differences in seedling form merely representing variations in the amount of cleavage. According to data presented, the cleavage may occur at various stages of development, extending possibly from the first division of the zygote to early growth of the seedling. The time of cleavage no doubt determines whether the resulting multiple seedling will be conjoined and how great a degree of attachment will persist in the seedlings. Cleavage that began in early embryonic development would probably have a greater opportunity to complete itself, producing separate but identical multiple seedlings. Cleavage in later stages would have less opportunity for completion and would more likely produce conjoined seedlings.

SUMMARY

Thirty-six lots of seed obtained from open- and cross-pollinated pistillate plants were sown. All multiple seedlings observed were grown for observation of their morphology, chromosome number, and genetic characters.

Of 405 multiple seedlings thus obtained, 97 per cent were twin seedlings, the remainder being higher multiples, mostly triplet seedlings. The frequency of polyembryony in seed lots varied from 0.13 to 3.54 per cent, with a mean of 0.95 per cent.

Diploids constituted 93.2 per cent of all plants derived from twin seedlings. The remainder consisted of triploids, trisomics, haploids, and tetraploids (in order of observed frequency). A seedling with a heteroploid number was always associated with a diploid seedling or with a seedling of the same heteroploid number. Diploids always exceeded haploids in seedling length. Aside from this group, however, relative seedling length was in no way related to chromosome number.

Nearly half of the multiple seedlings were morphologically attached in varying degrees. Such seedlings are designated as the conjoined type, the remaining multiple seedlings, the separate type. Normal seedlings having only one shoot and one primary root are referred to as the single type.

The plants derived from multiple seedlings of the separate type often differed cytogenetically. The distribution of sexes and stem color in twin seedlings reveals that about three fourths of the ovules producing them had each been penetrated by two pollen tubes. Certain evidence also indicates that twin embryo sacs may produce dizygotic twin seedlings. According to this information and to the analysis of combinations of chromosome numbers and of relative seedling lengths in twin seedlings, the remaining one fourth probably originate from cleavage of a single initial embryo.

The intrinsically double nature of the conjoined type, in contrast to that of the single type, is demonstrated by their rapid development into two independent plants. Judging from the complete identity in chromosome number and genetic characters between members of a conjoined twin seedling, attachment in nearly all cases results from incomplete cleavage of a single initial embryo. Most of the gross structural alteration involved in cleavage probably occurs during seed germination, although the process may also start in earlier stages of development.

VEGETABLE SEED PRODUCTION LABORATORY,
AGRICULTURAL EXPERIMENT STATION,
STATE COLLEGE OF WASHINGTON,
MOUNT VERNON, WASHINGTON
DIVISION OF TRUCK CROPS,
UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

LITERATURE CITED

- BRAUN, A. 1860. Über Polyembryonie und Keimung von *Coelebogone*. Abhandl. Akad. Wiss. Berlin Phys. Kl. 1859: 107-263.
- COOPER, D. C. 1943. Haploid-diploid twin embryos in *Lilium* and *Nicotiana*. Amer. Jour. Bot. 30: 408-413.
- FLORY, W. S., JR. 1932. Genetic and cytological investigations on *Asparagus officinalis* L. Genetics 17: 432-467.
- KAPPERT, H. 1933. Erbliche Polyembryonie bei *Linum usitatissimum*. Biol. Zentralblatt 53: 276-307.
- KASPARYAN, A. S. 1938. Haploids and haplo-diploids among hybrid twin seedlings in wheat. C. R. (Doklady) Acad. Sci. U. R. S. S. 20: 53-56.
- MÜNTZING, A. 1937. Polyploidy from twin seedlings. Cytologia Fujii Jub. Vol. 211-227.
- POPE, M. N. 1943. Cleavage polyembryony in barley. Jour. Hered. 34: 153-154.
- RICK, C. M., AND G. C. HANNA. 1943. Determination of sex in *Asparagus officinalis* L. Amer. Jour. Bot. 30: 711-714.
- SPRAGUE, G. F. 1932. The nature and extent of heterofertilization in maize. Genetics 17: 358-368.
- WEBBER, J. M. 1938. Cytology of twin cotton plants. Jour. Agric. Res. 57: 155-160.
- . 1940. Polyembryony. Bot. Rev. 6: 575-598.

THE EFFECT OF THE MOISTURE CONTENT OF THE SOIL UPON THE RATE OF EXUDATION¹

J. Joseph McDermott²

IT HAS long been known, in a qualitative way, that the moisture content of the soil surrounding the roots of a plant is an important factor in determining whether or not the root systems will show evidence of "root pressure" or exudation when the tops are removed. Lowry, Huggins, and Forrest (1936), after measuring sap flow from detopped corn plants concluded: "The rate of flow of sap varies with the amount of rainfall, where the total is less than that required to saturate the soil." More recently Kramer (1941), working with sunflowers and tomatoes, has shown that there is a definite point in the range of moisture content of soils between the moisture equivalent and the wilting percentage below which detopped root systems do not show exudation. This point, about midway between these two soil moisture contents, corresponds approximately to the moisture content below which the diffusion pressure deficit of the soil exceeds a value of one atmosphere. This study was undertaken to determine the relationship between the rate of exudation and the moisture content of the soil.

MATERIALS AND METHODS.—The soil used in this study was a uniform, fertile, sandy loam with a moisture equivalent of 20 per cent as determined by the centrifuge method. The wilting percentage was 5 per cent as determined with both wheat and sunflower seedlings.

A group of 4-inch pots was filled with soil and two sunflower seeds were sown in each pot. After germination, the seedlings were thinned out, leaving one healthy plant in each pot. The plants were about six weeks old and about 25 to 30 cm. high when used in the experiments.

During the afternoon of the day prior to that on which determination of exudation rates was to be made, a group of plants was brought in from the greenhouse and placed in a saturated atmosphere over night. This was done to minimize the effects which the tensions set up by transpiration might exert upon the exudation rates. The plants composing the group were selected at random from the available population. Within such a population individual plants differ in their total leaf-surface, rates of transpiration, and extent of root systems. To these

inherent variables must be added the possibility of some inequalities in the daily watering of the plants. Thus, with the possibility of different initial soil moisture contents, and different amounts of moisture lost by individual plants through transpiration, differences would be expected in the soil moisture contents of a series of plants selected at random from a population.

After the plants remained in the saturated atmosphere over night, their tops were removed about 4 cm. above the soil surface with a sharp razor. Pipettes were attached to the stumps by means of short pieces of close-fitting rubber tubing. The junctions of the tubing with the stump, and with the pipette were then made watertight by the application of a coating of modified grafting wax. The pipettes were partially filled with water and suction was applied for a short time to remove any air trapped in the stump, roots, or connections. The pots were placed in watertight galvanized iron containers and allowed to come to a temperature of 25°C. in a constant temperature bath. When temperature equilibrium was established, the level of the water in the pipettes was recorded as the initial reading and readings were taken hourly. Upon completion of the measurements, a sample of approximately 100 grams of soil was taken from each pot and all root fragments were removed. The samples were weighed and dried to constant weight at 105°C. The loss in weight on drying was taken to represent the amount of moisture present and was expressed as the percentage of the oven-dry weight of the soil.

RESULTS AND DISCUSSION.—In some of the earlier experiments, readings were taken for the first hour only, and in some of the later experiments readings were taken hourly for a period of four hours. To determine what difference might result in the correlation of soil moisture contents with the rates of exudation, using data for one hour only or for any of the other successive hours, or for the average of all the rates, the rates of exudation for four successive hours for eleven plants were subjected to a statistical examination.

The variation between plants was found to be significant, indicating that there is a real difference between the rates of exudation of the plants. However, the average decrease in rate of exudation was not significant, indicating that although on the average there was a decrease with time, this decrease did not occur consistently in all plants; the variations in behavior from plant to plant were sufficiently great that no one of the hourly readings for a given plant could be considered really different from that of any other hour. Therefore, the conclusion was reached that any one of the hourly rates or the average rate would give comparable results if correlated with the moisture content of the soil. Since the data

¹ Received for publication June 11, 1945.

² Chemist, Technical Collaboration Branch, Office of Foreign Agricultural Relations, United States Department of Agriculture.

The experimental work reported in this paper was done while the author was a graduate student in the Department of Botany, Duke University. The author wishes to acknowledge his indebtedness to Dr. Paul J. Kramer for assistance during the progress of this work and in preparation of the manuscript. He also wishes to acknowledge the assistance of Professor F. X. Schumacher of the School of Forestry, Duke University, and Dr. F. M. Wadley, Bureau of Entomology and Plant Quarantine in connection with the statistical treatment of the data.

of one series were complete for the first hour only, all calculations were based on first hour values.

The occurrence of negative rates of exudation, suggesting a loss of moisture to the soil surrounding the roots of the detopped plants, raised the question as to whether this water was actually lost to the soil or was merely used in lessening a water deficit in the root systems.

To obtain information on this point, a group of unwilted tomato plants, of an age comparable with that of the sunflower plants, was detopped and pipettes attached. The rate of negative exudation of these plants was observed for a period of 24 hours, after which the total amount of water lost from the pipettes was determined. The roots were removed from the soil and their volumes were measured by displacement of water. The data obtained are presented in table 1.

TABLE 1. *Total volume of water lost in 24 hours, volume of the root systems, and volume of water lost expressed as a percentage of the total volume of the root systems for six tomato plants.*

Volume of water lost (ml.)	Volume of roots (ml.)	Water lost	
		Root volume	× 100
0.80	4.50	17.8	
3.17	11.50	27.5	
3.88	5.90	65.7	
4.65	3.85	120.7	
2.96	2.30	128.7	
5.46	3.80	143.5	

It is quite impossible that a root system could absorb as much as 1.5 times its volume of water from the pipettes to make up any water deficit. Therefore, it is likely that most of the loss observed was a direct loss of water from the root system to the soil. It should be stressed that in no instance reported above was the soil moisture content as low as the wilting percentage.

Breazeale (1930) and Breazeale and Crider (1934) worked with entire plants, a portion of whose root systems were growing in soil with a moisture content below its wilting percentage. They found that the roots in the dry soil lost moisture to the soil resulting in a rise of the moisture content of the soil. Although the results given in table 1 are not strictly comparable to those obtained by Breazeale, an analogy may be drawn between them. It is generally recognized that at the wilting percentage the diffusion pressure deficit of the soil moisture approximately equals the diffusion pressure deficit of the moisture in the plant roots, and there is no longer a net movement of water into the roots from the soil. When the soil moisture content is below the wilting percentage, the gradient of diffusion pressure deficits is such that the net movement of water is from the plant to the soil. When the top is removed, that portion of the diffusion pressure deficit ascribable to the top is no longer transmitted to the roots. Since

both the rate and direction of water movement between root and soil are determined by their relative diffusion pressure deficits, the reduction of the diffusion pressure deficit of the roots caused by the removal of the tops shifts the moisture content below which water is lost to the soil from the permanent wilting percentage to a higher value.

In postulating the possible relationship between rate of exudation and moisture content of the soil several factors must be considered. From the osmotic standpoint one would expect, as a first approximation, that a hyperbolic relationship would fit the facts. As the moisture content of the soil approaches the permanent wilting percentage, the forces required to remove the remaining water increase greatly with each small increment of water removed; within the limits of experimental error, a perpendicular line passing through the value of the permanent wilting percentage appears to be asymptotic to the curve. In the other direction the forces required to remove the soil moisture become smaller and smaller with increasing water content, tending to approach zero force as a limiting value. It seems likely that if only the range of soil moisture contents between the permanent wilting percentage and the moisture equivalent were considered, the hyperbolic relationship might fit the facts rather well. However, when the moisture content of the soil exceeds the moisture equivalent (as happens in the field during and after heavy rains or irrigation) other factors come into play.

As the moisture content increases and the air-filled pore space decreases, aeration becomes progressively poorer. The concentration of oxygen decreases and the concentration of carbon dioxide increases, especially in soil containing numerous roots and microorganisms. Kramer (1940) has shown that when soil is saturated with carbon dioxide resistance to water movement through the roots increases materially and absorption of water, transpiration and exudation from detopped root systems are decreased. Saturating the soil with nitrogen produced much smaller changes in rate of these processes, indicating that lack of oxygen has less effect than an excess of carbon dioxide. Chang and Loomis (1945) also found water absorption to be materially reduced by a high concentration of carbon dioxide around the roots.

In view of these effects of carbon dioxide on the absorption of water it seems justifiable to assume that, above the moisture equivalent, increasing amounts of soil moisture will exert a depressing effect upon the rate of exudation due primarily not to excess moisture but to the corollary decrease in aeration. Therefore, when the entire range of possible soil moisture contents is considered, it seems that the relationship between the rate of exudation and the moisture content of the soil should be one which would rise from negative values, give positive values for the rates of exudation, and then after passing through a maximum, give reduced values for the rate of exudation.

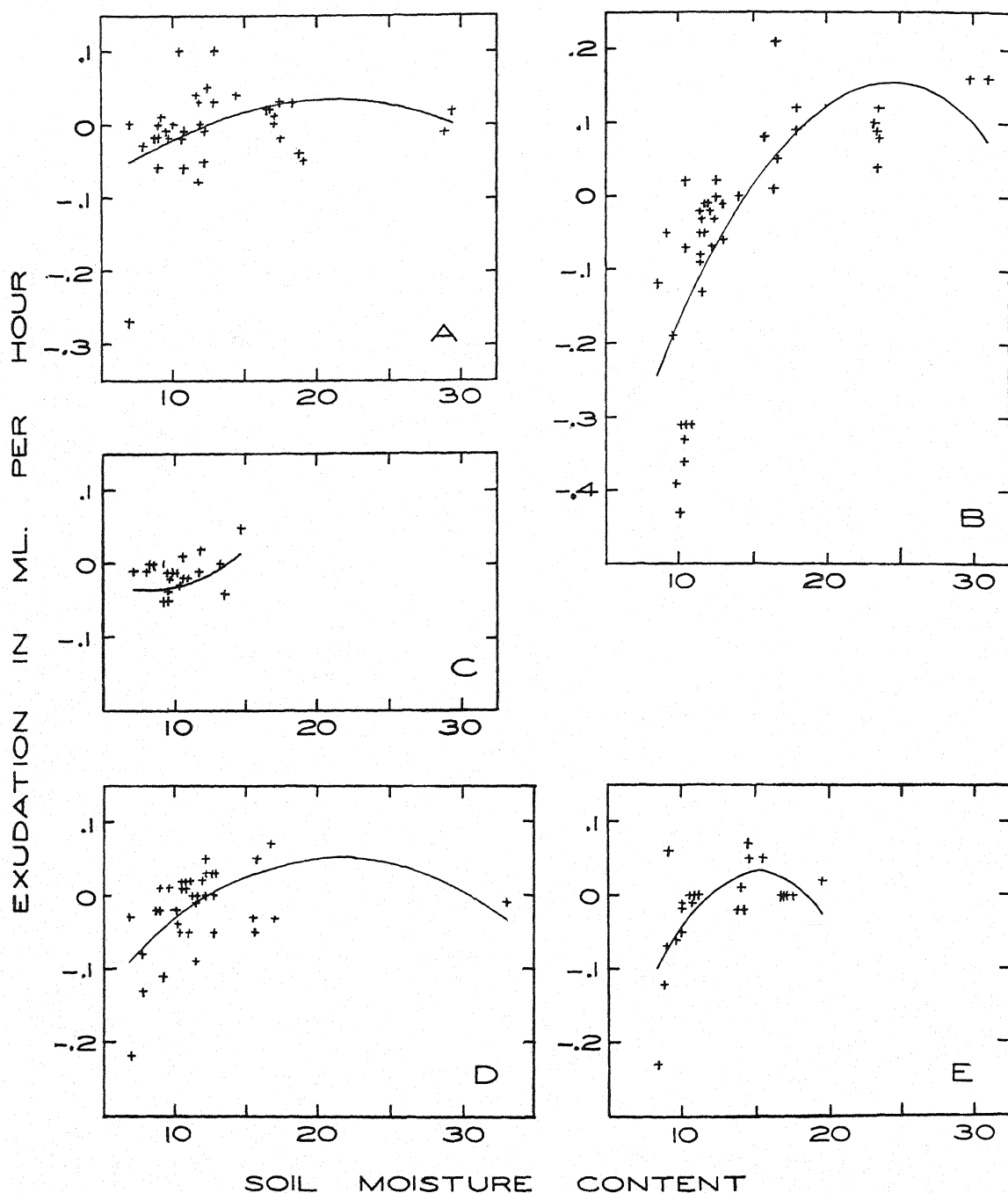


Fig. 1. The rates of exudation in ml. per hour are plotted against their respective soil moisture contents for each series of determinations. The curve shown for each series is the parabola of best fit as determined by the method of least squares. The equations for these curves can be found in table 2.

The rates of exudation plotted against their corresponding soil moisture contents are shown in figure 1 for each of the five series. The best fitting curves derived by the least squares technique are also shown for each series of data. In the preliminary calculations hyperbolas, parabolas, and logarithmic curves were calculated but it was established that

the parabolas shown were the best fitting of these three curve-types.

In figure 2 is shown the curve which incorporates the data from all five of the series. The derived equations for all six curves are given in table 2.

For each of the series an analysis of variance was calculated and the contribution of each of the two

TABLE 2. Equations for the relation between soil moisture content and rate of exudation.^a

Series		Number of plants
A	$Y = -0.157 + 0.017 \chi - 0.0004 \chi^2$	36
B	$Y = -0.797 + 0.078 \chi - 0.002 \chi^2$	42
C	$Y = +0.015 - 0.015 \chi + 0.001 \chi^2$	24
D	$Y = -0.232 + 0.026 \chi - 0.0006 \chi^2$	36
E	$Y = -0.637 + 0.089 \chi - 0.003 \chi^2$	24
Total regression	$Y = -0.249 + 0.025 \chi - 0.0005 \chi^2$	162
Without series C	$Y = -0.292 + 0.029 \chi - 0.0006 \chi^2$	138

^a Y = the rate of exudation in ml. per hour and x = the moisture content of the soil in percentage on the dry basis.

variables to the sum of squares was tested for significance. The resulting F values are tabulated in table 3.

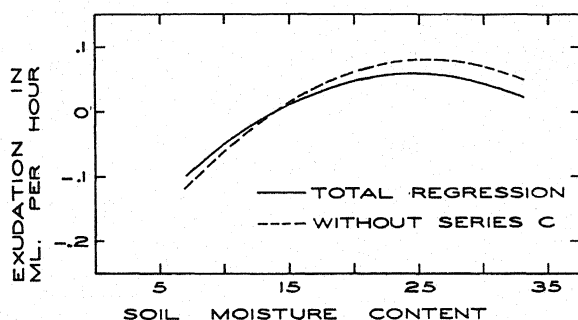


Fig. 2. Average relationship between the rate of exudation and the moisture content of the soil. The solid line represents all of the data obtained, while the broken line does not include the results of series C. The equations for these curves can be found in table 2.

The F values tabulated in table 3 are the figures necessary for the statistical evaluation of the derived relationship between the rate of exudation and the soil moisture content. The values which exceed those necessary for significance indicate that there is a real relationship between the rate of exudation and the soil moisture content. The values attributed to b_1 are those which consider only the linear relationship between the rate of exudation and the soil moisture contents. Those values for b_2 deal with the relationship between the square of the moisture content and the rate of exudation. An examination of the table reveals that for the regression representing all of the data, there is a highly significant linear relationship between the rate of exudation and the

soil moisture content (b_1 significant) and that the part which may be ascribed to the relationship between the rate of exudation and the square of the soil moisture content is not significant. This indicates that for all the data presented a straight line relationship between the rate of exudation and the soil moisture content is sufficient representation.

Equations calculated for the five series of data which constitute the basis for the total equation show characteristics somewhat different from the average. For example, series C, consisting of the measurements of a series of 24 plants, shows no relationship at all in either the linear or parabolic terms. This fact seems to be due mainly to the limited scope of the sample, for series E, although representing the same number of plants, has wider distribution of values and presents a better sample for regression analysis. In series A and D the linear relation is not significant, but in B and E this is highly significant. In series A, B, D, and E, the parabolic relationship is significant. Therefore, since four of the five series of observations point to the presence of a significant parabolic relationship, it seems possible that the short range of soil moisture of series C might be responsible for the failure of the total regression to show a significant parabolic relationship. This possibility was investigated by calculations of the F values attributable to b_1 and b_2 when the results of series C were excluded. These are 33.95 and 7.58, both of which exceed the F value of 6.82 derived from the table which corresponds to 1 per cent level of significance.

From the curve of figure 2, the moisture content of the soil when the rate of exudation was zero was obtained. This value of 14 per cent was used to calculate the portion of the range of soil moisture from

TABLE 3. Values of F calculated for the regression coefficients of the equations of table 2.

Series	A	B	C	D	E	
Source of variation						Total
b_1	2.41 ^a	38.33 ^c	1.86 ^a	3.33 ^a	9.38 ^c	35.47 ^c
b_2	18.62 ^c	8.82 ^c	...	10.13 ^c	6.35 ^b	2.05 ^a

^a Not significant.

^b Significant beyond the 5 per cent level.

^c Significant beyond the 1 per cent level.

the wilting percentage (5 per cent) to the moisture equivalent (20 per cent) which was unavailable for exudation to the detopped root systems of sunflowers. A total of 60 per cent of the soil moisture available to the whole plant when the soil is at the moisture equivalent is not available to the detopped root systems. This result is somewhat different from Kramer's (1941), which showed for three very different types of soil, a coarse sand, a sandy loam, and heavy clay, that the lower 45 per cent of the range of moisture content from the wilting percentage to the moisture equivalent is unavailable to detopped root systems for exudation. It confirms in general, however, his observations and also those made by Lowry, Huggins, and Forrest (1936) on the effect of soil moisture on exudation.

The occurrence of a significant relationship between the rate of exudation and the square of the soil moisture content does not necessarily imply that this is a direct effect of soil moisture. As previously stated, it may result from the effects of moisture content on soil aeration. The results of this study indicate that soil moisture in the higher part of the range depresses the rate of exudation from detopped root systems, probably because it decreases the oxygen content and increases the carbon dioxide content of the soil.

SUMMARY

Measurements of the rate of exudation of detopped root systems of sunflower plants grown in a sandy loam soil were made and the soil moisture content was determined for five groups of sunflower plants totaling 162 plants.

Within a range of moisture content just above the wilting percentage of the soil, the detopped root systems lose water to the surrounding soil, exhibiting "negative exudation." With increased soil moisture, the rate of "negative exudation" decreases, becomes zero, and then positive exudation begins, increasing to a maximum at about the moisture equivalent. Soil moisture contents higher than the moisture equivalent result in a decrease in the rate of exuda-

tion which is probably due to the poorer aeration, especially the high CO_2 content, which accompanies any further increase of soil moisture content.

On the average, exudation stopped at 14 per cent soil moisture. From this result calculations show that the lower 60 per cent of the soil moisture in the range from the wilting percentage to the moisture equivalent is unavailable to detopped root systems of sunflowers.

An equation of the parabolic type was derived which expresses the relationship between the rate of exudation and the soil moisture content and the square of the soil moisture content for each of the five series of observations and for the five series taken together.

Four of the five series show a significant relationship between the square of the soil moisture content and the rate of exudation; two show significant relationships between the soil moisture content and the rate of exudation. One series showed no significant relationship at all.

ESTACION EXPERIMENTAL AGRICOLA,
DEL ECUADOR

LITERATURE CITED

- BREAZEALE, J. F. 1930. Maintenance of moisture-equilibrium and nutrition of plants at and below the wilting percentage. *Arizona Exper. Sta. Tech. Bull.* 29.
- , AND F. J. CRIDER. 1934. Plant association and survival, and the build-up of moisture in semi-arid soils. *Arizona Exper. Sta. Tech. Bull.* 53.
- CHANG, H. T., AND W. E. LOOMIS. 1945. Effect of carbon dioxide on absorption of water and nutrients by roots. *Plant Physiol.* 20: 221-232.
- KRAMER, PAUL J. 1940. Causes of decreased absorption of water by plants in poorly aerated media. *Amer. Jour. Bot.* 27: 216-220.
- . 1941. Soil moisture as a limiting factor for active absorption and root pressure. *Amer. Jour. Bot.* 28: 446-451.
- LOWRY, M. W., W. C. HUGGINS, AND L. A. FORREST. 1936. The effect of soil treatment on the mineral composition of exuded maize sap at different stages of development. *Georgia Agri. Exper. Sta. Bull.* 193.

CELL NUMBER IN SUCCESSIVE SEGMENTS OF AVENA COLEOPTILES OF DIFFERENT AGES: MATERIAL FOR THE BIOCHEMIST ¹

G. S. Avery, Jr., Margaret Piper, and Patricia Smith

DATA ON auxins, enzymes, vitamins, etc., are commonly expressed in terms of weight of the tissue in which they are contained. Such units are arbitrary, and bear little or no relation to the units comprising the living organism. The structural and functional unit of the organism is, of course, the cell; and with the advance of micromethods in biochemistry it seems time for a serious attempt to express physiological data concerning multicellular organisms on a per-cell basis. This requires actual counting of cells. The *Avena* coleoptile is a good object with which to begin; its structure is simple (approximately 95 per cent of its volume consisting of undifferentiated cells), and much is already known of its physiology.

The object of this report is to present actual numbers of cells in consecutive segments of entire *Avena* coleoptiles of different ages. It is presented chiefly as a reference work for those who may wish to study and express biochemical data in higher plants on a per-cell basis. Living coleoptiles are a convenient material for such biochemical studies because they provide readily available fresh material. They can be imbedded in low melting point paraffin, the paraffin thoroughly chilled in ice water, and sections of fresh tissue cut at 75 to 250 μ , depending upon the age of the tissues. These sections can then be freed from paraffin, the enclosed section of embryonic foliage leaf removed, and the remaining coleoptile segment containing a known number of cells used for biochemical studies. This method was employed by Avery and Linderström-Lang (1940) for a study of peptidase activity in *Avena* coleoptiles.

MATERIALS AND PREPARATION.—Seeds of *Avena sativa* L. var. Victory were obtained from Mr. Marshall Rumsey of Batavia, New York; only seeds weighing between 30 and 33 mg. were used in this study. These were germinated in petri dishes on moist filter paper at a temperature of 24–25°C., receiving only phototropically inactive red or yellow light during germination. The petri dishes were tilted in such a way that the coleoptiles grew approximately straight upward from the seed. When coleoptiles plus elongated first internodes measured 1.5, 4, 10, and 17 mm. in length, the coleoptiles were carefully excised from the seedlings and fixed in Navashin's fluid. Actual lengths of coleoptiles are given in table 1. Twenty of each of the four lengths of coleoptiles were dehydrated in an ethanol series, cleared in chloroform, and imbedded in paraffin. Ten of each length were cut in serial transections at 25 μ , and another ten were cut in serial longisections at 15 or 20 μ . The number of coleoptiles finally employed for cell counts, etc., is given in table 1. The length measurements given in this table are derived by multiplying the number of sections by 25 μ (thickness

of sections); these computed lengths usually checked to within 0.1 mm. of the length of the median longisections.

TABLE 1. *Lengths and numbers of coleoptiles employed for determining number of cells at the several stages of growth.*

Length mm.	Number of coleoptiles	
	Transections	Longisections
1.5	10	10
3.8	10	9
8.3	9	9
14.0	9	8

DETERMINATION OF CELL NUMBER.—*Transection cell counts.*—In the 1.5 mm. coleoptiles, cells in each 25 μ transection were counted under low or high power of the microscope with the aid of a hand tally counter. Counts of every four successive sections were then averaged, the result being the mean number of cells (in transection) in a given 100 μ segment of the coleoptile. The counts were then averaged for the corresponding segments of ten coleoptiles. The variation in cell number between corresponding segments of different coleoptiles was rarely more than 1 per cent. Cell counts in older coleoptiles were made in the same way, except that after the first 1000 μ (the distal ten segments of 100 μ each), counts were made of four successive transections at intervals of 100 to several hundred microns apart. Graphs were plotted and the points interpolated for segments where actual counts were not made.

Cell lengths.—Median longisections of coleoptiles served for cell-length measurements. The cells are arranged in longitudinal rows, with all walls transverse—hence cell-length measurements were relatively easy to make, and dependable. In each 1.5 mm. coleoptile, cell length was determined for 20 cells in each 100 μ of length. These data were averaged for the ten coleoptiles. In the longer coleoptiles, intervals were skipped, after the distal 1000 μ , as in the transection cell counts. Average cell lengths throughout the coleoptile were then graphed and a smooth curve drawn. This procedure seems to suggest that there was considerable fluctuation in length measurements, whereas except for an occasional "off" point, the raw data curves were almost smooth. From such smoothed curves, cell lengths were taken for use with the transectional cell counts in the calculation of number of cells per segment.

Epidermal cells.—In the 1.5 mm. coleoptile, epidermal cells were included in all transection counts and cell-length measurements. It has been shown previously that in the growth of the coleoptile, epidermal cells elongate but do not divide (Avery and

¹ Received for publication June 27, 1945.

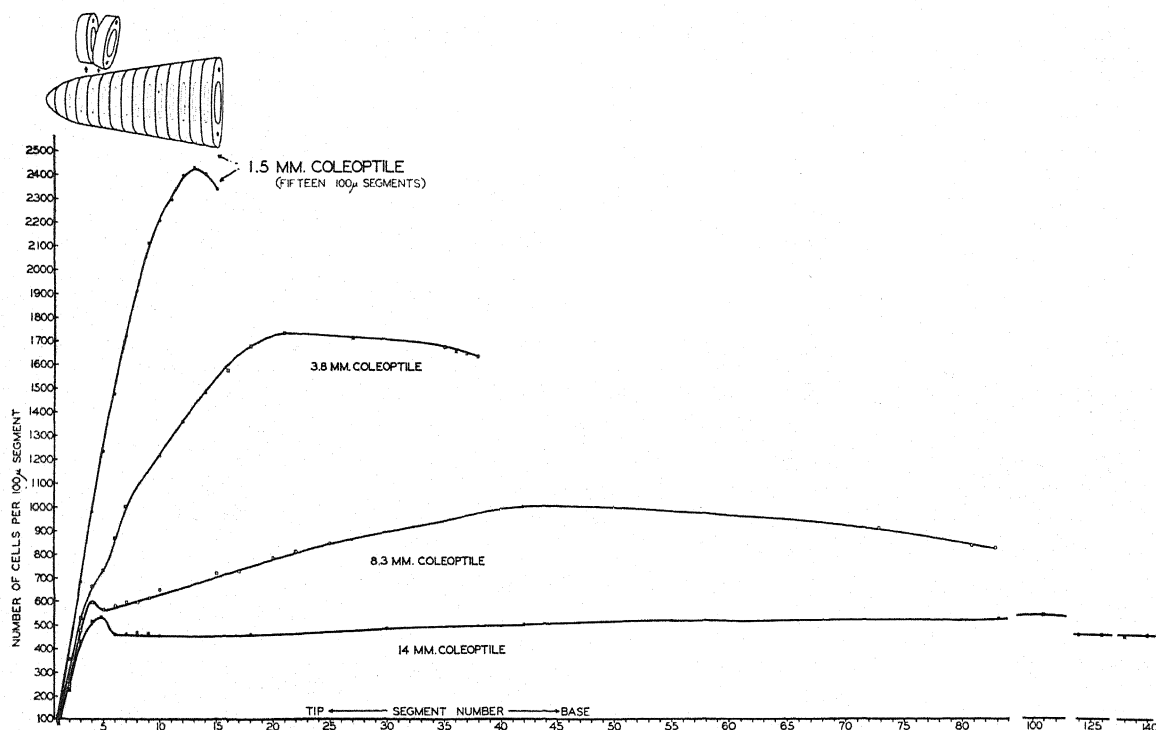


Fig. 1. Number of cells per segment in *Avena* coleoptiles of different ages (lengths). Points represent actual determinations. Diagram (upper left) indicates relative size of $100\ \mu$ segments in 1.5 mm. coleoptile; embryonic foliage leaf ordinarily enclosed in coleoptile not shown in diagram.

Burkholder, 1936). This means that epidermal cell length in older coleoptiles is many times that of the underlying cells; hence for the 3.8-mm. and longer coleoptiles, epidermal cell counts and lengths were kept separate and subsequent calculations carried out separately. In the distal 0.4–0.5 mm. of the coleoptile, epidermal cells do not elongate, so need not be determined separately, even in older coleoptiles.

Vascular bundles.—Because of difficulties inherent in determining the number of cells in vascular bundles, these were omitted from this study. The bundles were calculated to make up approximately 5 per cent, on the average, of the volume of the coleoptile. The error involved is thus relatively small and relatively constant.

Calculation.—For the sake of simplicity in calculating averages, etc., cell-length measurements were kept in ocular micrometer units; not until final calculations were made were they converted to microns. Final calculations were made as follows:

Given average cell length of $61.5\ \mu$ for a particular segment, and transectional cell number of 286 (epidermal cells excluded), the number of cells in a $100\ \mu$ segment of coleoptile would be $100 \div 61.5 \times 286 = 465$. If the same $100\ \mu$ segment shows 132 epidermal cells in transection, and the average epidermal cell length in this segment is $160\ \mu$, then the total number of epidermal cells is $100 \div 160 = 0.625 \times 132 = 83$; and the total number of cells in

the segment is therefore $465 + 83 = 548$. The curves in figure 1 were constructed from such calculations. Table 3 indicates which values are original and which interpolated. The interpolated figures (table 3) are taken from the curves in figure 1.

RESULTS.—Growth of the coleoptile, and total number of cells involved.—Except for segments 1 to 4 in coleoptiles of all ages, there is a marked decrease in number of cells per segment as coleoptiles grow longer (fig. 1). This is due chiefly to cell elongation, also to decreasing rate of cell division as the coleoptile elongates. The total calculated number of cells in coleoptiles of the lengths studied, appears in table 2. It may be seen that the total number of cells increases up to the 8.3 mm. stage. From there on, cell division does not occur and growth is due entirely to cell elongation. This is substantially the same result as that reported earlier (Avery and Burkholder, 1936), although obtained by a different method.

TABLE 2. Total number of cells in coleoptiles of different ages (vascular bundle cells omitted). See figure 2.

Coleoptile length mm.	Number of cells (nearest round number)
1.5	24,700
3.8	53,800
8.3	71,000
14.0	69,400

Number of cells in 100 μ segments—coleoptiles of different ages.—Table 3 presents all the data obtained in this study from actual counts and meas-

TABLE 3. Total number of cells, exclusive of vascular bundle cells, in successive 100 μ segments of *Avena* coleoptiles of different ages. Figures in bold face are actual determinations, others derived by interpolation.

Segment no. (beginning at tip)	1.5	3.8	8.3	14
1	118	106	93	86
2	358	253	245	227
3	686	531	480	431
4	983	666	599	518
5	1235	737	567	535
6	1477	872	583	463
7	1724	1004	599	465
8	1915	1159	599	471
9	2111	1190	615	465
10	2209	1220	655	457
11	2298	1290	650	457
12	2399	1364	663	457
13	2434	1422	678	458
14	2407	1486	693	458
15	2347	1532	726	459
16	...	1578	722	459
17	...	1630	734	460
18	...	1682	750	461
19	...	1701	765	462
20	...	1720	790	463
21	...	1740	796	465
22	...	1737	817	467
23	...	1733	825	469
24	...	1730	838	473
25	...	1727	851	476
26	...	1724	856	478
27	...	1721	856	480
28	...	1717	878	482
29	...	1713	886	484
30	...	1709	896	487
31	...	1704	907	489
32	...	1700	916	491
33	...	1696	926	493
34	...	1687	935	495
35	...	1682	941	497
36	...	1662	955	498
37	...	1654	965	499
38	...	1641	975	500
39	985	501
40	994	495
41	1000	503
42	1006	506
43-49	1008	509
50	999	512
51-54	993	516
55	986	520
56-61	974	520
62-65	956	521
66-68	942	521
69-72	924	522
73	913	522
74	900	522
75	883	523
76-79	880	523
80	860	523

TABLE 3. Continued.

Segment no. (beginning at tip)	1.5	3.8	8.3	14
81	845	523
82	842	523
83	835	524
84-87	528
88-93	540
94-100	547
101	548
102-107	544
108-109	530
110-112	516
113-116	500
117-119	482
120-123	465
124	461
125	462
126	464
127-137	460
138	458
139	460
140	462

TABLE 4. Total number of cells, exclusive of vascular bundle cells, in successive 75 μ segments of 1.5 and 3.8 mm. *Avena* coleoptiles. Data calculated from those presented in table 3.

Segment no. (beginning at tip)	Distance of basal end of segment below tip of coleoptile (μ)	Coleoptile (mm.) 1.5	3.8
1	75	60	70
2	150	190	140
3	225	345	260
4	300	548	400
5	375	692	470
6	450	868	540
7	525	990	590
8	600	1112	640
9	675	1298	690
10	750	1394	750
11	825	1490	800
12	900	1588	840
13	975	1660	885
14	1050	1705	925
15	1125	1752	970
16	1200	1804	1010
17	1275	1830	1046
18	1350	1816	1080
19	1425	1795	1120
20	1500	1765	1150
21	1575	...	1180
22	1650	...	1210
23	1725	...	1240
24	1800	...	1260
25-27	1290
28-33	1300
34-38	1290
39-44	1275
45-48	1260
49-51	1240

TABLE 5. Total number of cells, exclusive of vascular bundle cells, in successive 125 μ segments of *Avena* coleoptiles of different ages (lengths). Data calculated from those presented in table 3.

Segment no. (beginning at tip)	Distance of basal end of segment below tip of coleoptile (μ)	Coleoptile (mm.)			
		1.5	3.8	8.3	14
1	125	200	170	160	140
2	250	610	450	430	380
3	375	1080	680	720	600
4	500	1480	900	720	660
5	625	1910	1120	720	580
6	750	2250	1260	750	584
7	875	2540	1395	760	584
8	1000	2740	1510	810	573
9	1125	2900	1630	836	573
10	1250	3020	1730	860	575
11	1375	3020	1820	886	575
12	1500	2950	1900	910	575
13	1625	1970	915	575
14	1750	2040	920	575
15	1875	2100	950	575
16	2000	2140	990	578
17	2125	2175	1005	580
18	2250	2175	1020	580
19	2375	2170	1045	585
20	2500	2160	1060	590
21	2625	2155	1070	595
22	2750	2150	1070	598
23	2875	2140	1085	600
24	3000	2130	1100	610
25	3125	2125	1120	610
26	3250	2115	1140	610
27	3375	2105	1155	615
28	3500	2090	1175	615
29	3625	2070	1190	615
30	3750	2060	1210	615
31	3875	1230	618
32	4000	1245	620
33	4125	1250	625
34-36	1260	633
37-38	1255	638
39-42	1245	643
43-44	1233	650
45-47	1215	653
48-50	1200	660
51-53	1185	665
54-55	1165	665
56-59	1148	666
60-61	1122	670
62-63	1192	670
64	8000	1070	670
65-66	1050	670
67-82	678
83-84	670
85-86	655
87-89	640
90-92	625
93-95	605
96-98	585
99-112	575

urements. It may be seen that cell number for many segments was obtained by interpolation.

For workers contemplating micro-studies such as

that previously reported by Avery and Linderström-Lang (1940), it is important to know that living coleoptiles from 1.5 to 14 mm. in length may be cut

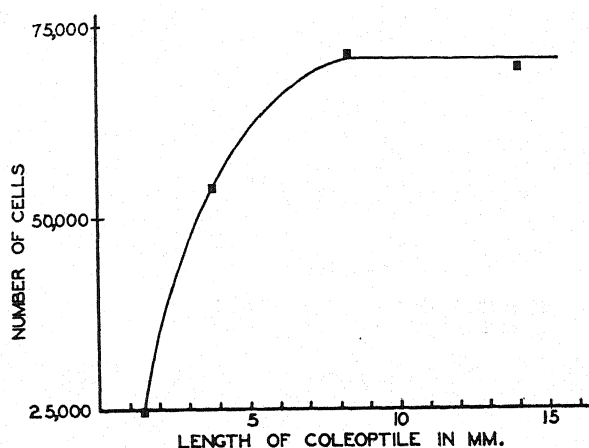


Fig. 2. Total number of cells in *Avena* coleoptiles at various stages of elongation (exclusive of vascular bundle cells).

into 100 μ segments of fresh tissue. Some difficulty may be encountered with 14 mm. coleoptiles, however, even with a sharp microtome blade.

Number of cells in 75 μ and 125 μ segments from coleoptiles of different ages.—Tables 4 and 5 contain data for 75 μ and 125 μ segments, all calculated from table 3. In numerous instances the accuracy of the calculations was checked against the original counts and measurements. Those who may wish to use the data for 75 μ segments should know that it has been possible to cut only 1.5 and 3.8 mm. coleoptiles at this thickness. The fresh tissue of coleoptiles of these ages is sufficiently firm to hold together and make clean slices without tearing. Fresh segments of coleoptiles 10 mm. or more in length are more readily cut at 125–250 μ . For this reason data on 125 μ segments are presented also (table 5). From combinations of cell counts in segments of these three thicknesses, the number of cells in segments of other thicknesses can be determined.

DISCUSSION.—The coleoptiles were all grown in a darkroom at 24 to 25°C., in a saturated or near-saturated atmosphere. Although in darkness most of the time, they were examined periodically under phototropically inactive yellow light. Anyone plan-

ning to use the data presented here in connection with biochemical studies, should for sake of accuracy employ similar conditions for growth of *Avena* seedlings (as well as the same variety).

Tables 4 and 5, giving numbers of cells in 75 and 125 micron coleoptile segments, are included only for ease of reference. In some instances, if the columns in these tables are added, it will be noted that they do not exactly agree with totals appearing in table 2. These differences, from coleoptile to coleoptile, depend chiefly upon any "left over" fragment after the lowermost complete segment (such as the 50 μ fragment below the thirtieth 125 micron segment of the 3.8 mm. coleoptile).

As to duration of cell division in the coleoptile: the data in table 2 show that there is no increase in cell number after the coleoptile is approximately 8 mm. in length. This is in full agreement with the earlier report of Avery and Burkholder (1936), in which it was concluded that "most of the increase in numbers of cells takes place by the time the coleoptile is 1 cm. long. . ."

SUMMARY

The total number of cells, exclusive of vascular bundle cells, is reported for successive segments of etiolated *Avena* coleoptiles of different ages. Data are presented for segments 75, 100, and 125 μ in thickness, cut from coleoptiles 1.5, 3.8, 8.3, and 14 mm. long. Living coleoptiles may be imbedded in paraffin and sectioned at these thicknesses for enzyme or other determinations; hence the cell numbers recorded here may be employed wherever there is reason to express biochemical data on a per-cell basis in any part of the coleoptile.

BROOKLYN BOTANIC GARDEN,
BROOKLYN, NEW YORK

LITERATURE CITED

- AVERY, G. S., JR., AND P. R. BURKHOLDER. 1936. Polarized growth and cell studies on the *Avena* coleoptile, phytohormone test object. Bull. Torrey Bot. Club 63: 1-15.
—, AND K. LINDERSTRÖM-LANG. 1940. Peptidase activity in the *Avena* coleoptile, phytohormone test object. Bot. Gaz. 102: 50-63.

BRAZILIAN CHYTRIDS. VII. OBSERVATIONS RELATIVE TO SEXUALITY IN TWO NEW SPECIES OF SIPHONARIA¹

John S. Karling

SEXUAL REPRODUCTION in the monocentric eucarpic rhizidiaceous chytrids appears to be comparatively rare, or at least it has been observed in only a few of the known species. In the majority of these chytrids the resting spores have been reported to develop asexually without prior fusion of gametes. However, more than forty years ago in Denmark, Petersen (1903) found a monocentric chytrid, *Siphonaria variabilis*, in insect exuviae the resting spores of which appeared to have been formed by fusion of the contents of two small thalli through anastomosed rhizoids. He reported and figured the presence of a small empty thallus attached by a tube or rhizoid to the mature spore and stated that sexuality was probably involved in the development of the resting spores of this species. He reaffirmed this statement later in 1909 and 1910. The observations of Petersen on *S. variabilis* were substantiated by Sparrow in 1935, who further reported that the same type of sexual reproduction occurs in *Rhizoclostridium* also. He confirmed these reports in 1937 and expressed the belief that a similar fusion of thalli would likewise be found in *Asterophlyctis*. The discovery by the author in Brazil and in the United States of two additional species of *Siphonaria* in which the resting spores appear to be formed sexually confirms in general the observations of Petersen and suggests that the type of sexual reproduction exhibited by these species may possibly be widespread in certain genera of chytrids.

Petersen distinguished *Rhizoclostridium*, *Asterophlyctis*, and *Siphonaria* on the basis of differences in the structure of the rhizoidal system and sporangia. The sporangia of the first genus were described as globose, smooth, apophysate, and subtended by relatively fine rhizoids. *Asterophlyctis* was reported to be similar to *Rhizoclostridium* except for its somewhat stellate and spiny sporangia and resting spores. *Siphonaria*, on the other hand, was described as nonapophysate with thick-walled, tube-like rhizoids, and because of the latter characteristic Petersen named the genus *Siphonaria*. These generic distinctions, based on vegetative structural differences, were subsequently recognized and accepted by most chytridologists. The report by Sparrow (1937, p. 34), however, that *Siphonaria* may sometimes be apophysate and the discovery by the author of a nonapophysate *Asterophlyctis*-like species with occasional tube-like rhizoids show that the presence of an apophysis and the nature of the rhizoids in these species are variable characters and of doubtful importance in distinguishing genera. The report that all the genera are characterized by the same type of

sexual reproduction is of greater significance in phylogeny and classification and should take precedence over variable vegetative character in diagnosis. In the event Sparrow's observations on *Rhizoclostridium* and *Asterophlyctis* are confirmed, these genera should be merged with *Siphonaria* on the grounds of similarity of type of sexual reproduction. *Asterophlyctis sarcopoides*, however, has been isolated frequently by the author and grown in pure culture on chitin and chitin agar, and so far no evidence of fusion of thalli has been observed. It is therefore questionable whether or not *Asterophlyctis* can be merged with *Siphonaria* on this basis.

The species found by the author in Brazil are named *S. petersenii* and *S. sparrowii*, respectively, in honor of Drs. H. E. Petersen of Denmark and F. K. Sparrow, Jr., of the University of Michigan, who introduced and extended the study of chytrids which inhabit the exuviae of insects. Relatively few specimens of the Brazilian species were found in an unidentified insect case at Flores Nabuco near Manaus, Amazonas, but after the author had returned to the U.S.A., he found an abundance of both species in the exuviae of mayflies near Pawling, New York. As was noted earlier, the first of these species is *Asterophlyctis*-like, with spiny but nonapophysate sporangia, while the second one is similar to the type species, *S. variabilis*. The present observations relate entirely to living material, but a further study of plasmogamy, karyogamy, and meiosis from fixed and stained material is now in progress and will be described in a later paper.

SIPHONARIA petersenii sp. nov.—Fungus saphrophyticus; sporangia extramatriculibus, spinosis, colore areo-luteo, longitudine pyriformibus, $5-20 \times 10-36 \mu$; zoosporis sphericis, $3-3.5 \mu$; sporis perdurantibus sphericis, $10-15 \mu$, ovatis vel angularibus, $6-8 \times 10-14 \mu$.

Thalli numerous, occurring inside of empty insect exuviae. Sporangia extramatricul to substratum, non-apophysate, orange-golden in color, predominantly elongately pyriform, $5-20 \times 10-36 \mu$, occasionally elongated transversely to rhizoid axis, with a long sharp apical and 3-12 lateral, simple or bifurcate spines, $4-15 \mu$ in length; exit papilla subapical, low and inconspicuous. Zoospores spherical, $3-3.5 \mu$, with a small (1.5μ) golden-red globule; swarming in a vesicle for several minutes before breaking out and swimming away. Rhizoidal system intramatricul, monoaxial, arising from base of sporangium, richly branched and extending for a distance of 80μ . Resting spores spherical, $10-15 \mu$, oval, or slightly angular and spiny, $6-8 \times 10-14 \mu$, with a reddish-brown (2μ), slightly uneven, crusty and almost verrucose wall; containing numerous granules and globules; formed sexually by the fusion of the contents of one or more "male" (?) thalli

¹ Received for publication June 29, 1945.

This work has been greatly facilitated by a Grant-in-Aid of Research from the Graduate Faculties of Columbia University.

with a "female" (?) thallus; germination unknown.

Saprophytic inside the exuviae of mayflies and other insects, Flores Nabuco, Amazonas, Brazil; Pawling, New York, and Candlewood Lake, Connecticut, U.S.A.

SIPHONARIA sparrowii sp. nov.—Fungus saprophyticus; sporangii extramatrixlibus, non-apophysatis, hyalinis, levibus, sphericis, 8–30 μ , vel pyriformibus; zoosporis sphericis, 5–5.6 μ ; sporis perdurantibus sphericis, 10–18 μ , levibus, hyalinis ad fulvum colorem; rebus contentis emergentibus in germinatione et zoosporangium superficiale formantibus.

Thalli numerous, occurring inside insect exuviae. Sporangia extramatrixlibus to substratum, non-apophysate, hyaline, smooth, spherical, 8–20 μ , or broadly pyriform with a low apical or subapical exit papilla. Zoospores spherical, 5.5–6 μ , with a large (3–3.5 μ) hyaline refractive globule; swarming in a vesicle outside the sporangium for several minutes before breaking out and swimming away. Rhizoidal system monoaxial, intramatrixlibus, arising from base of sporangium, rhizoids thick-walled, coarse, tapering abruptly, and often branching at right or obtuse angles. Resting spores oval, spherical, 10–18 μ , with a thick (3–4 μ) amber to dark brown smooth wall; several spores frequently enveloped by a common thick wall; formed sexually by fusion of the content of one or more "male" thalli with one "female" thallus; functioning as prosperangia in germination.

Saprophytic inside exuviae of mayflies, Flores Nabuco near Manaus, Amazonas, Brazil, and Sharon, Connecticut, U.S.A.

Siphonaria petersenii and *S. sparrowii* occur inside the empty insect cases, and when the latter are mounted and studied *in toto*, the chytrids look as if they were completely intramatrixlibus. However, such is not the case. The sporangia are extramatrixlibus and project into the cavity of the empty case, while the rhizoids are intramatrixlibus and spread out in the inner layers of the integument. At maturity the zoospores are liberated within the empty exuviae, where they swim about and later germinate. Only rarely have the sporangia of either species been observed on the exterior surface of the empty insect cases. Apparently both species are chitinophyllic like *Rhopalophlyctis* and *Chytriumyces*, recently described by the author (1945). At present *S. petersenii* appears to be more variable than *S. sparrowii* in relation to chitinous substrata, since it has been found in exuviae of midges and other insects in addition to those of mayflies. It has also been isolated on pure shrimp chitin and grown on chitin-agar media. *Siphonaria sparrowii*, on the other hand, has never been observed for certain on any substrata besides the exuviae of mayflies; nor has it been isolated on shrimp chitin and chitin agar. Chitin from mayflies is now being purified, and it is quite probable that with such a substratum *S. sparrowii* may be isolated and grown in pure culture.

Vegetative development and asexual reproduction.

—The structure and developmental cycles of the

vegetative phases of *S. petersenii* and *S. sparrowii* are shown in figures 1 to 14 and 27 to 41, respectively, and inasmuch as they do not differ fundamentally from those of other similar chytrids, it is not necessary to describe these processes in detail. The length of the vegetative phase varies considerably in the two species. In *S. petersenii* a large number of successive generations of sporangia and zoospores occur before resting spores are formed, whereas in *S. sparrowii* comparatively few sporangia have been observed so far. There is, nevertheless, a distinct cyclic alternation of sporangia and resting spores, which may possibly represent the monoploid and diploid generations, respectively.

The zoospores of *S. petersenii* which include a golden or orange-red globule (fig. 1) swim about 30 to 90 minutes, come to rest (fig. 2), and germinate (fig. 3). The germ tube branches (fig. 4) and gives rise to the intramatrixlibus system of rhizoids (fig. 5–7). At the same time, a small bud develops at the apex of the spore body (fig. 4) and elongates into a terminal spine (fig. 5–8). Before it attains full length, however, the lateral spines begin to develop in the same manner (fig. 7) and reach their ultimate dimensions by the time the sporangia are mature (fig. 8–13). The number of spines vary from 3 to 12 per sporangium and may attain a length of 15 μ . They are usually simple and sharply pointed, but occasionally bifurcated ones occur (fig. 9, 10). The majority project upward at an acute angle from the surface of the sporangium, but some spines may extend outward at right angles or even project downward (fig. 8, 9, 13, 14).

The globule in the germinating zoospore retains its golden color for some time, but as its fragments or its material becomes dispersed, the color changes to light orange and gradually disappears. By the time the incipient sporangia have attained the size shown in figures 7 and 8, the protoplasm appears to be almost hyaline. These changes in pigmentation are very similar to those described by Sparrow (1937) for *S. variabilis*. Later, as the refringent material begins to coalesce to form the definitive globules of the zoospores, the pigmentation reappears, so that by the time cleavage begins the globules are deeply golden red in color.

The coarseness, length, and branching of the rhizoidal system varies with the size of the sporangia. The branches are relatively fine for the vegetative thalli, but in the case of the zygotes or resting spores (fig. 20, 21) they become almost as thick-walled and tubular in appearance as those of *S. variabilis* and *S. sparrowii*. The exit papilla usually develops subapically (fig. 11, 13, 14) and fairly close to the apical spine and is comparatively low and inconspicuous.

Shortly after the completion of cleavage and the development of the zoospore rudiments, the exit papilla deliquesces. The zoospores ooze out slowly in a globular mass (fig. 13) which soon becomes enveloped by a delicate hyaline membrane. Within a short time the zoospores begin to move slowly

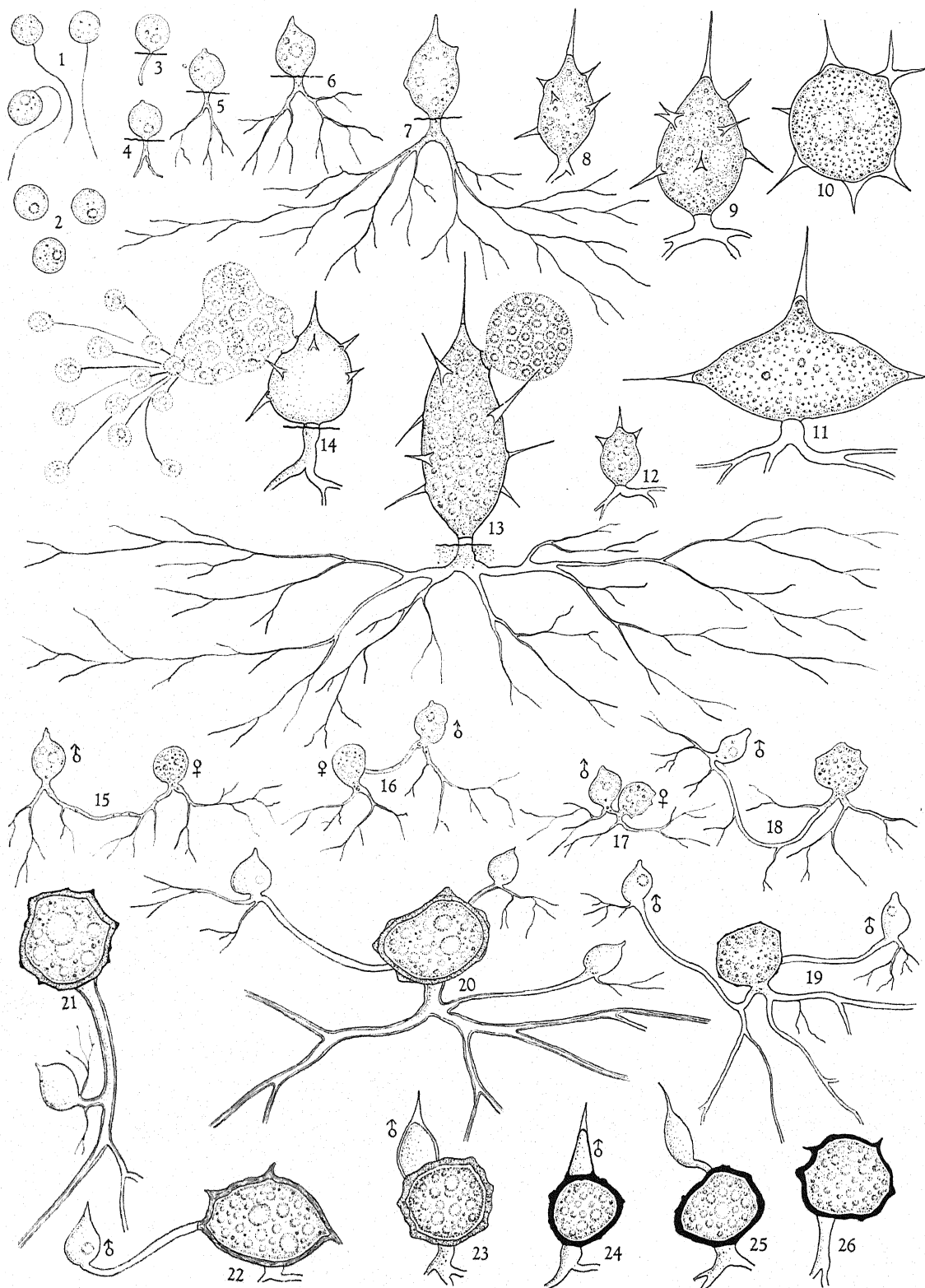


Fig. 1-26. *S. petersenii*.—Fig. 1. Zoospores. $\times 1600$.—Fig. 2. Zoospores at rest. $\times 1600$.—Fig. 3-6. Germination stages, establishment of thalli, and origin of apical spine. $\times 1600$.—Fig. 7. Young thallus with two incipient lateral spines. $\times 1600$.—Fig. 8, 9. Slightly older thalli with fully developed apical and lateral spines. $\times 1700$.—Fig. 10. Cross section of sporangium. $\times 1600$.—Fig. 11. Flattened sporangium with subapical exit papilla. $\times 1600$.—Fig. 12. Minute sporangium

within the vesicle, and then in a few seconds they begin to swarm rapidly. This violent swarming causes the vesicle to change rapidly and constantly in shape in the same manner described by the author (1944b) for species of *Rhizidium*. That the vesicle is attached to and continuous with the sporangium is shown by the ease with which the zoospores pass from one structure to the other during the swarming stage. This phase usually lasts for several minutes, and when the vesicle ruptures, the zoospores escape (fig. 14) and disperse rapidly. It is to be noted, however, that if the dehiscence of the sporangium and emergence of the zoospores are premature or abnormal, no membrane appears to form, and the zoospores separate without swarming. Under such conditions they may become feebly motile, but they usually die and disintegrate in a short while.

The color of the protoplasm, position of the exit papilla, presence of elongate sharply-pointed spines on the sporangia, a vesicular membrane around the emerged spore mass, and the discharge of the zoospores within the empty insect case distinguish this species sharply from *A. sarcoptoides*, so that there is no doubt about their being distinct species. In *A. sarcoptoides*, according to Sparrow (1937), the exit papilla or tube usually occurs at the base of the sporangium, and the zoospores are discharged to the outside of the exuviae. So far this has never been observed in *S. petersenii*. Sparrow was unable to demonstrate the presence of a membrane around the discharged spore mass in *A. sarcoptoides*, but its presence and development in *S. petersenii* appear to be normal as far as present observations go.

The development of *S. sparrowii* (fig. 27-41) is essentially the same as that of *S. petersenii* with the exception that no spines are formed on the sporangia. Furthermore, the rhizoids of the vegetative thalli are not so richly branched but coarser, more abruptly tapering and tube-like, and may often form branches at right or obtuse angles (fig. 34, 35). The Brazilian species differs sharply from *S. variabilis* by its larger hyaline zoospores, lack of reddish-brown or golden globules in the protoplasm, position of the exit papilla, presence of a membrane around the discharged spore mass, and by the lack of a "nose" or blunt papilla at the base of the sporangium. In addition, the sporangia do not become apophysate so far as is now known. Otherwise, *S. sparrowii* is similar in structure and development to the type species. The sporangia may occasionally be quite small and form only two or four zoospores (fig. 36, 37). As the apical or subapical exit papilla deli-

quesces, the zoospores ooze out slowly (fig. 39) and normally become enveloped by a vesicular membrane. The subsequent behavior of the zoospores, their swarming and passage from vesicle to sporangium or vice versa, rupture of the vesicle, and the escape of the zoospores from the latter structure (fig. 40) are similar to those described above for *S. petersenii*. In this species also the vesicular membrane may fail to develop if dehiscence of the sporangium is premature or abnormal.

Syngamy.—Resting spore or zygote formation appears to be comparatively rare in *S. petersenii*, as far as present studies go, and has been observed in only five collections of exuviae from Candlewood Lake, Connecticut. In *S. sparrowii*, on the other hand, it occurs in great abundance so that several hundreds of resting spores are usually present in a single infected insect case. In contrast, the formation of zoosporangia seems to be sparse and even rare, and to date relatively few sporangia have been found. The resting spores of both species, nevertheless, appear to develop as the result of fusion of the contents of minute thalli which are usually isomorphic. These thalli apparently arise from motile cells which come to rest, germinate, and form a sparse rhizoidal system. Up to a certain stage they usually develop in the same manner as the vegetative thalli and are very similar to them in structure and appearance. Figure 15 shows two such thalli of *S. petersenii*, the rhizoidal systems of which are joined by a narrow canal or tube. In this species one of the fusing thalli is usually surmounted by a peg or spine, while the other is generally more globular and smooth (fig. 15, 16). These differences, however, are not always so marked as in these two figures. Inasmuch as the content of the spiny thallus flows into the smooth one, the former is designated as male and the latter as female.

The thalli in figure 15 are joined by their rhizoids, but in figure 16 the fusion tube or rhizoid from the male connects directly with the body of the female thallus. The latter condition is quite common, as is shown in figures 19, 20, 22-25. In figure 17, on the other hand, the thalli are connected at the base by a short tube. In the early developmental stages the protoplasm of the two thalli is very similar in density and appearance, but within a short time after the thalli become connected, that of the female appears denser and more coarsely granular with several refringent bodies. The male thallus at this stage generally contains a large refractive globule in addition to the less optically heterogeneous proto-

with three spines. $\times 1600$.—Fig. 13. Mature thallus showing character of the rhizoidal system and the discharge of zoospores from the exit papilla. $\times 1200$.—Fig. 14. Escape of the zoospores from the vesicular membrane. $\times 1200$.—Fig. 15. Two thalli with rhizoids connected by a narrow tube; the so-called male thallus is topped by a short spine. $\times 1600$.—Fig. 16. Two fusing thalli with the fusion tube attached directly to the main body of the female thallus. $\times 1600$.—Fig. 17. Fusing thalli which are attached and continuous at their bases. $\times 1700$.—Fig. 18. Young developing zygote with one attached male thallus. $\times 1700$.—Fig. 19. Later developmental stage of a zygote with two attached male thalli. $\times 1700$.—Fig. 20. Full sized warty zygote with three empty male thalli; rhizoids coarse and comparatively thick-walled. $\times 1500$.—Fig. 21. Mature warty zygote. $\times 1500$.—Fig. 22. Spiny zygote connected with a male thallus by a broad tube. $\times 1500$.—Fig. 23-25. Empty spiny male thalli attached directly to zygotes. $\times 1500$.—Fig. 26. Parthenogenic (?) resting spore. $\times 1500$.

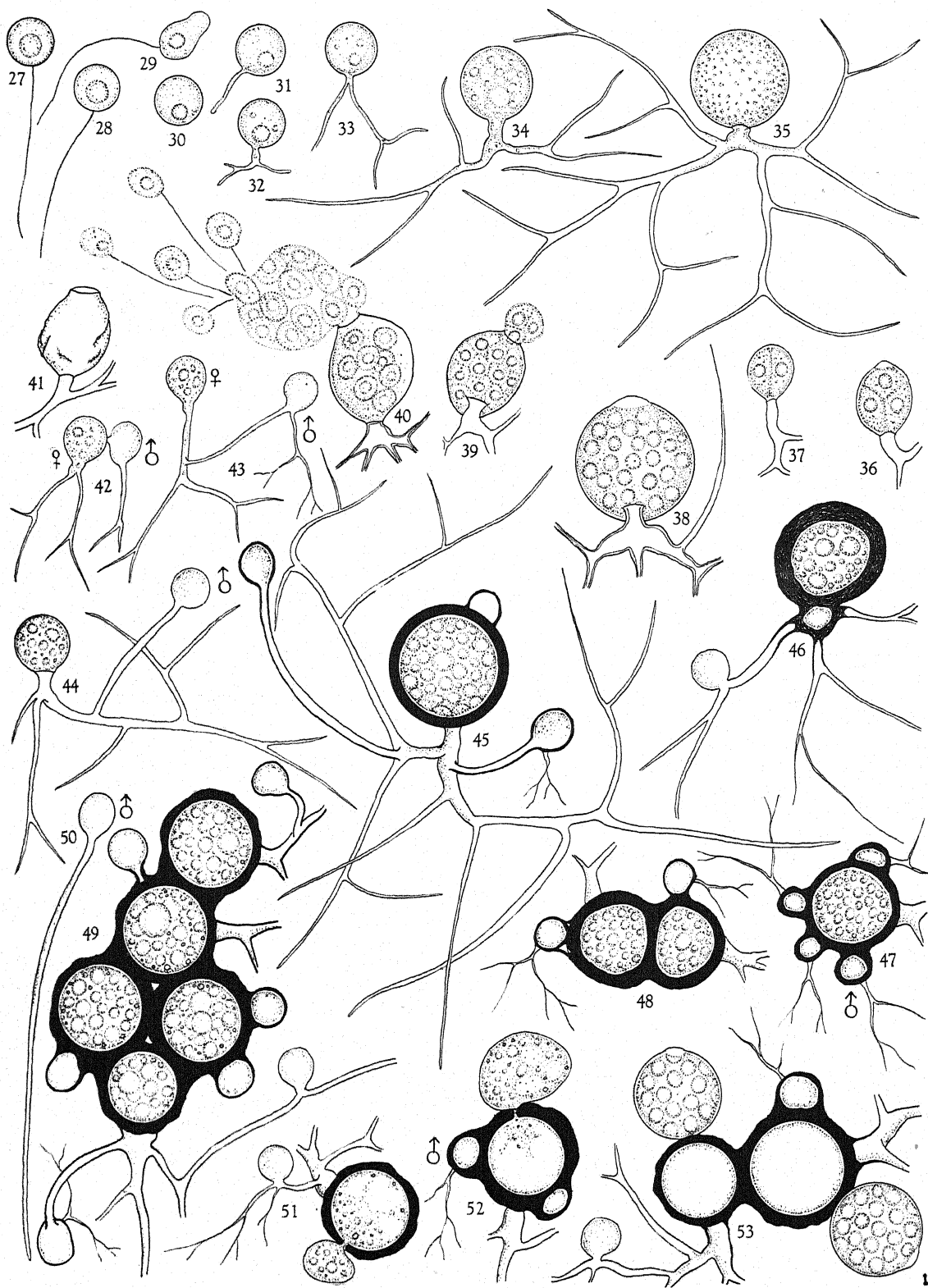


Fig. 27-53. *S. sparrowii*.—Fig. 27-29. Spherical and amoeboid zoospores. $\times 1600$.—Fig. 30. Zoospore at rest. $\times 1600$.—Fig. 31-33. Germination stages. $\times 1600$.—Fig. 34, 35. Young and almost mature thalli with spherical sporangia and characteristically branched rhizoids. $\times 1200$.—Fig. 36, 37. Minute, mature sporangia. $\times 1200$.—Fig. 38. Sporangium shortly before dehiscence. $\times 1000$.—Fig. 39, 40. Emergence of zoospores and their escape from vesicle. $\times 900$.—Fig. 41.

plasm. These differences in appearance are probably associated with the initial stages of syngamy. Movement of the protoplasm from the male to the female thallus has apparently begun in figures 15, 16, and 17. The content of the incipient zygote in figure 17 is considerably denser and more refractive, and the surface of the spore is slightly irregular at one side. A later stage is shown in figure 18 in which the male thallus is almost empty except for a large refractive globule. The incipient zygote has almost doubled in size, and its protoplasm has become denser and more granular. In addition, its rhizoids have become more extensive and branched. The latter structures increase in diameter, length, and extent of branching as development proceeds, and absorb the nutriment for the growing zygote. In some mature stages of resting spore formation the rhizoids of *S. petersenii* may become almost as large, tube-like, and thick-walled as those of *S. variabilis* and *S. sparrowii*, which indicates that this character is not always generically distinctive.

A later stage of zygote development is shown in figure 19 in which two male thalli have fused with one female. This is not uncommon in *S. petersenii*, and in a few cases four empty male thalli were found attached to a mature zygote. The fusion tube of one of the male thalli in figure 19 is connected directly with the incipient resting spore, while that of the other one is joined with a rhizoid. The zygote in this figure is slightly angular in shape and continuous with the rhizoids, and its wall has begun to thicken and turn brown in color. As the zygote matures, it is delimited from the rhizoids by a cross wall which thickens and becomes part of the spore wall. Further advanced maturation stages are shown in figures 20 and 21. In the former figure two of the empty male thalli are connected with the zygote and the third is joined to the rhizoidal system. The walls of the zygotes in these two figures are fairly thick, reddish-brown in color, and slightly crusty or warty, and in other cases the warts may be prolonged into short spines (fig. 22, 26). The content of the maturing zygote is usually granular with numerous large refractive globules (fig. 20, 23), but by the time the spores are fully mature, the globules usually appear smaller in size and more numerous (fig. 24-26).

As was noted earlier, the male thalli usually develop a reduced rhizoidal or absorbing system (fig. 15-21) which ceases to develop after fusion. In a few cases observed, however, no rhizoids were visible, and the male thalli were joined to the zygote by a fairly broad canal or tube (fig. 22, 25). In other instances, the spiny and conical male thalli were attached directly to the zygotes without an intervening conjugation tube (fig. 23, 24). The close relation

to the respective thalli in these figures suggests that the male gamete may possibly have come to rest on the female thallus, after which fusion occurred without any previous development of rhizoids or a long conjugation canal. A few resting spores were found which were devoid of attached male thalli (fig. 26), and it is quite probable that they had developed parthenogenetically. It is obvious from the above description that the extent of development of the male thallus and its spatial relation to the female varies considerably in *S. petersenii*.

The same degree of variation has also been observed in *S. sparrowii* (fig. 42-53). In this species, however, no structural differences have been found in the so-called male and female thalli. They are both globular in shape and equal in size, with smooth walls, and the only basis of distinction is the movement of the germplasm from one into the other. In this species also the thalli which are to fuse develop like the vegetative plants, and up to a certain stage it is almost impossible to determine what their function will be. Figure 42 shows a male and female thallus after the completion of syngamy. In this case fusion apparently occurred through a short tube between the main bodies of the thalli as in some species of *Rhizophidium* (Sparrow, 1933, p. 520), instead of through the rhizoids. This close relation between the main bodies of the conjugating thalli doubtless explains the frequent attachment of empty male vesicles to the mature zygotes as shown in figures 47, 49, 52, and 53. Fusion of the protoplast through anastomosed rhizoids or a long conjugation tube also is very common in *S. sparrowii*. Figure 43 shows an empty male thallus with one of the rhizoids in direct continuity with the rhizoidal axis of the incipient zygote. A later stage is shown in figure 44 in which rhizoids *per se* are lacking at the base of the empty male thallus. The male thallus in this case is connected with the rhizoid of the developing zygote by a relatively broad tube. The same is true of the male thallus at the left in figure 45, but in this case the conjugation tube is longer and slightly broader. In *S. variabilis*, Sparrow (1937) reported that in many cases contact between thalli which fuse "appeared to have been accomplished solely by the efforts of the thallus which was later to become the resting spore," or, in other words, the so-called female thallus. In *S. sparrowii* the contact often seems to occur fortuitously, but in a large number of cases observed the male thallus appeared to grow toward the female. In several instances a male thallus without rhizoids was found which had developed a long tube toward a distant, previously fertilized female. The distance between the two, however, was so great that the protoplasm of the male appears to

Empty wrinkled sporangium. $\times 1000$.—Fig. 42. Completion of fusion; main bodies of gametic thalli connected by a short tube or papilla. $\times 1400$.—Fig. 43, 44. Later stages; tubes or rhizoids from empty thallus continuous with rhizoidal axis and rhizoids of incipient zygotes. $\times 1400$.—Fig. 45. Full grown zygote with thick brown wall and three attached empty male thalli. $\times 1400$.—Fig. 46. Zygote with empty male cell attached to rhizoid. $\times 1400$.—Fig. 47. Zygotes with four directly attached empty male thalli which are enveloped by the zygote wall. $\times 1400$.—Fig. 48. Two zygotes with fused walls. $\times 1400$.—Fig. 49. Five zygotes enveloped by a common wall. $\times 1400$.—Fig. 50. Abortive male (?) thallus with long tube; rhizoids absent. $\times 1400$.—Fig. 51, 52. Germination stages of zygote. $\times 1400$.—Fig. 53. Two germinated empty zygotes with thin-walled sporangia on their surface. $\times 1400$.

have been exhausted before the tube reached the female, and no contact was established. Figure 50 shows such a thallus which the author interprets as male. While this is not absolutely certain, the fact that it resembles the one at the left in figure 45 and also grew toward a clearly defined female thallus, suggests, at least, that it is male. Male thalli of the type shown in figure 50 usually become brown in color and fairly thick-walled with age.

The number of empty male thalli attached to a mature zygote varies from one to six. Only one is present in figures 48, 51, and 53, but in figure 47 four are visible. Whether or not the contents of several males fuse with that of one female is not certain yet, since the successive stages of multiple syngamy have not been observed in detail. It is not improbable, however, that multiple fusion of protoplasts occurs fairly often in this chytrid.

After syngamy, the young zygote increases rapidly in size, and its rhizoids become more extensive, branched, and coarse (fig. 44). Those of the male thallus, on the other hand, do not develop further but may often become brown and slightly coarser and look as if thickening of the walls had occurred. As the zygote matures, its wall thickens very markedly and becomes dark brown in color. This thickening and coloration may often extend to the conjugation tube and male thallus as shown in figure 45. If the latter is closely adherent to the zygote, it usually becomes enveloped by and incorporated in the zygote wall and appears as a thick-walled empty appendage to which a few rhizoids may be attached (fig. 47, 48, 52, 53). The wall of the zygote may often become 4 μ thick, and when several zygotes lie in close association, the walls usually fuse. Figures 48 and 53 show pairs of resting spores with confluent walls, while in figure 49 five spores have been enveloped by the thickening walls. This is a common occurrence in *S. sparrowii*, and in several cases as many as eight joined zygotes were observed.

The zygotes germinate readily under laboratory and field conditions after a dormancy of two to four weeks. In this process they function as prosperangia like the resting spores of most chytrids. A pore is first formed in the thick wall, after which the protoplasm slowly emerges (fig. 51) and develops into a thin-walled hyaline zoosporangium (fig. 52, 53) on the surface of the spore. These sporangia give rise to posteriorly uniflagellate motile cells which, as far as present observations go, appear similar to those produced by the primary sporangia (fig. 40).

In this connection it may be noted that the type species, *S. variabilis*, also has been found in abundance in the exuviae of mayflies and other insects. This species has been isolated on pure shrimp chitin and transferred to chitin agar. The structure and development of the vegetative thalli, zoosporangia, zoospores, and zygotes of the Brazilian material are very similar to those described by Sparrow (1937) for the Danish and North American specimens. In spite of the abundance of material collected and cultured on chitin media, however, zygotes developed only rarely.

DISCUSSION.—In analyzing the data relative to resting spore formation in *Siphonaria* presented above, serious consideration must be given to the possibility that the anastomosis of and fusion through rhizoids or long tubes between thalli relate to vegetative fusions instead of sexual reproduction. In other chytrids such as *Cladochytrium* (Berdan, 1941) and *Nowakowskiella* (Butler, 1907; Karling, 1944a) vegetative anastomosis of rhizoids occurs fairly often, and fusions of hyphae without subsequent zygote development is a common occurrence in higher fungi. In *Siphonaria petersenii* and *S. sparrowii*, on the other hand, fusion of the contents of thalli by means of anastomosed rhizoids or tubes is almost invariably associated with resting spore formation. For this reason, the author interprets these fusions as syngamous, although karyogamy has not yet been observed.

However, much remains to be learned before final conclusions can be drawn. So far it is not known at what stages in the life cycle karyogamy, meiosis, and sex segregation occur, if multiple fusions occur, whether the so-called gametes are uni- or multinucleate at the time of plasmogamy, or the exact origin of the thalli which fuse. On the grounds that such thalli develop in the same manner as and are similar in appearance and structure to those which give rise to zoosporangia, it is assumed that they originate from motile posteriorly uniflagellate cells of the same type as the zoospores.

It is quite probable also that karyogamy takes place shortly after plasmogamy, and, as in most other fungi, meiosis probably occurs at the first division of the diploid nucleus during germination of the zygote. In that event, the vegetative thalli, zoosporangia, zoospores and gametes are monoploid, and *S. petersenii* and *S. sparrowii* are typical haplonts. If sex is genotypically determined at meiosis in the germinating zygotes, the sporangia from the latter (fig. 43) will produce an equal number of male and female motile cells which germinate and develop into male and female thalli. Then, after a period of successive generations of such thalli, sporangia and motile cells, fusion of gametic thalli begins in the manner described above. Accordingly, the life cycles of *Siphonaria petersenii* and *S. sparrowii* would follow the pattern shown in diagram 1. In this relation it is to be noted that no structural differences have been observed in the successive generations of thalli, sporangia and zoospores which follow zygote germination, and if these thalli are of separate sexes, it is obvious that the male and female strains in *Siphonaria* are morphologically alike.

If, on the other hand, meiosis occurs during germination of the zygote but sex is phenotypically determined at the close of the sporangial and zoosporic period, as has been suggested for *Synchytrium* and *Olpidiopsis*, *Siphonaria* would have the alternate life cycle shown in diagram 2.

These suggested alternate life cycles are, of course, hypothetical, and determination of their accuracy must await further cytological and culture studies. Nevertheless, the data at hand indicate, in

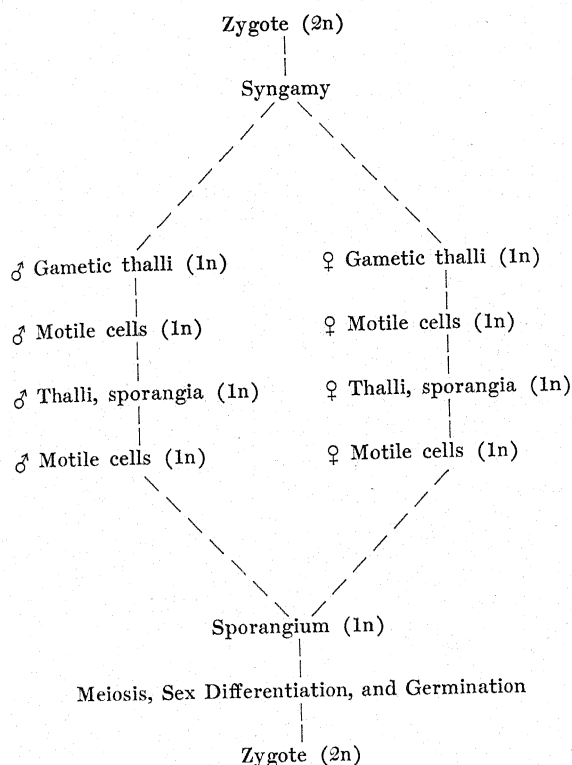


Diagram 1. Showing hypothetical life cycle of *Siphonaria* based on the supposition of genotypic sex segregation at meiosis in the germinating zygote.

the author's opinion, that sexual reproduction is isogamous and suggestive in many respects of that

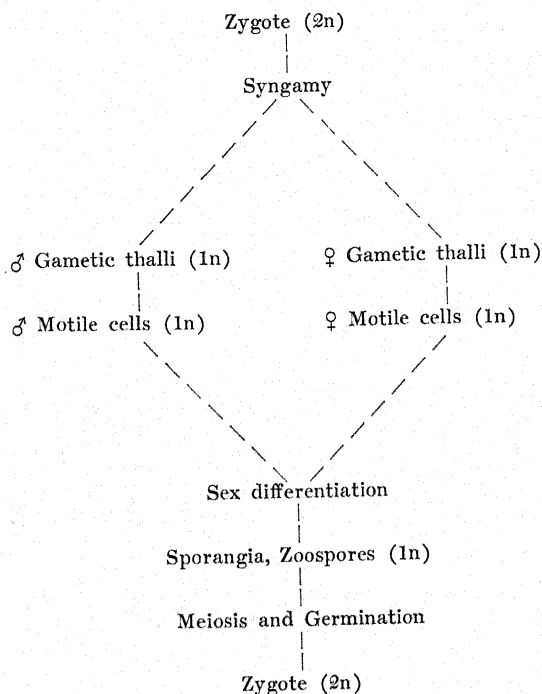


Diagram 2. Showing hypothetical life cycle of *Siphonaria* based on the supposition of phenotypic sex segregation at the close of the sporangial and zoosporic period.

in most Zygomycetes. As was noted earlier, the thalli which fuse are usually isomorphic, although the so-called male thallus in *S. peterseii* is often topped by a spine. In neither species, however, are differentiated gametes formed in the thalli prior to fusion, and, as far as present observations go, the protoplasts appear to fuse without undergoing visible structural changes. Therefore, it is a moot question whether these thalli are to be designated as differentiated gametangia or gametes. Consequently, until additional data are available, the author has designated the fusing thalli by the noncommittal descriptive name of gametic thalli.

SUMMARY

Siphonaria peterseii and *S. sparrowii* occur as saprophytes in insect exuviae in Brazil and the U.S.A. Present observations indicate that the resting spores develop as the result of union of minute isomorphic thalli through anastomosis of rhizoids or conjugation tubes. After union has been established, the content of one thallus flows into the body of another, after which the fused protoplasts develop into the zygote. Frequently, more than one of the so-called male thalli fuse with the female. In germinating, the zygote of *S. sparrowii* gives rise to a thin-walled sporangium which produces posteriorly uniflagellate cells. Although no accurate chromosome counts have been made, observations on living material suggest that these species of *Siphonaria* have well-marked alternations of monoploid and diploid generations.

DEPARTMENT OF BOTANY,
COLUMBIA UNIVERSITY,
NEW YORK CITY

LITERATURE CITED

- BERDAN, H. B. 1941. A developmental study of three saprophytic chytrids. I. *Cladochytrium hyalinum*. Amer. Jour. Bot. 28: 422-438.
- BUTLER, E. J. 1907. An account of the genus *Pythium* and some Chytridiaceae. Mem. Dept. Agric. India 1(5): 1-160.
- KARLING, J. S. 1944a. Brazilian chytrids. I. Species of *Nowakowskiella*. Bull. Torrey Bot. Club 71: 374-389.
- . 1944b. Brazilian chytrids. II. New species of *Rhizidium*. Amer. Jour. Bot. 31: 254-261.
- . 1945. Brazilian chytrids. VI. *Rhopalophlyctis* and *Chytriomycetes*, two new chitinophyllic operculate genera. Amer. Jour. Bot. 32: 362-369.
- PETERSEN, H. E. 1903. Note sur les Phycomycetes observés dans les teguments vides des nymphes de Phryganées avec description de trois espèces nouvelles de Chytridiaceae. Jour. de Bot. 17: 214.
- . 1909. Studier over Ferskvands—Phykomyceter. Bot. Tidssk. 29: 345-429.
- . 1910. An account of Danish fresh-water Phycomycetes with biological and systematic remarks. Ann. Mycol. 10: 494-560.
- SPARROW, F. K. 1933. Inoperculate chytridiaceous organisms collected in the vicinity of Ithaca, N. Y., with notes on other aquatic fungi. Mycologia 25: 513-535.
- . 1935. Recent contributions to our knowledge of the aquatic Phycomycetes. Biol. Rev. 10: 152-186.
- . 1937. Some chytridiaceous inhabitants of submerged insect exuviae. Proc. Amer. Philos. Soc. 78: 23-53.

STUDIES IN THE DEVELOPMENTAL ANATOMY OF PHLOX DRUMMONDII HOOK. I. THE EMBRYO¹

Helena A. Miller and Ralph H. Wetmore

THE FUNDAMENTAL background of our knowledge of the organization of vascular plants was established by the classical studies of Nägeli (1858), Sachs (1868), Hanstein (1868, 1870), Russow (1872), etc. They established the broad basis of plant anatomy in both embryonic and adult stages. During the sixty years following 1870, their basic tenets were not radically changed. In the past fifteen years, numerous descriptive and interpretative studies on the gymnosperms and angiosperms have added much to our knowledge of the orderly sequence of events during development. However, no single investigation, so far as the authors know, has followed the complete developmental story of an angiosperm from the fertilization of the egg through

¹ Received for publication July 2, 1945.

² In this study, the terms *embryonic*, *embryology*, etc., refer to *embryos* only, not to developmental stages in the stem and root apices.

to the mature plant. The present study has therefore been directed at the major changes in developmental anatomy throughout the entire life cycle of a single species; the results are treated under three headings: the embryo, the seedling, and the apices of the mature plant.

The embryonic stages² of many species have been studied in the last one hundred years. Some of these studies have been concerned with cell lineage in the early stages; others have dealt with developmental anatomy in the late stages of the embryo; only a few have reported on both cell lineage and developmental anatomy. Part I of this investigation deals with both aspects of development in the ontogeny of *Phlox*, covering progressive changes from the fertilization of the egg to the maturation of the embryo in the seed.

MATERIALS AND METHODS.—The species selected for study was *Phlox Drummondii* Hook. This spe-

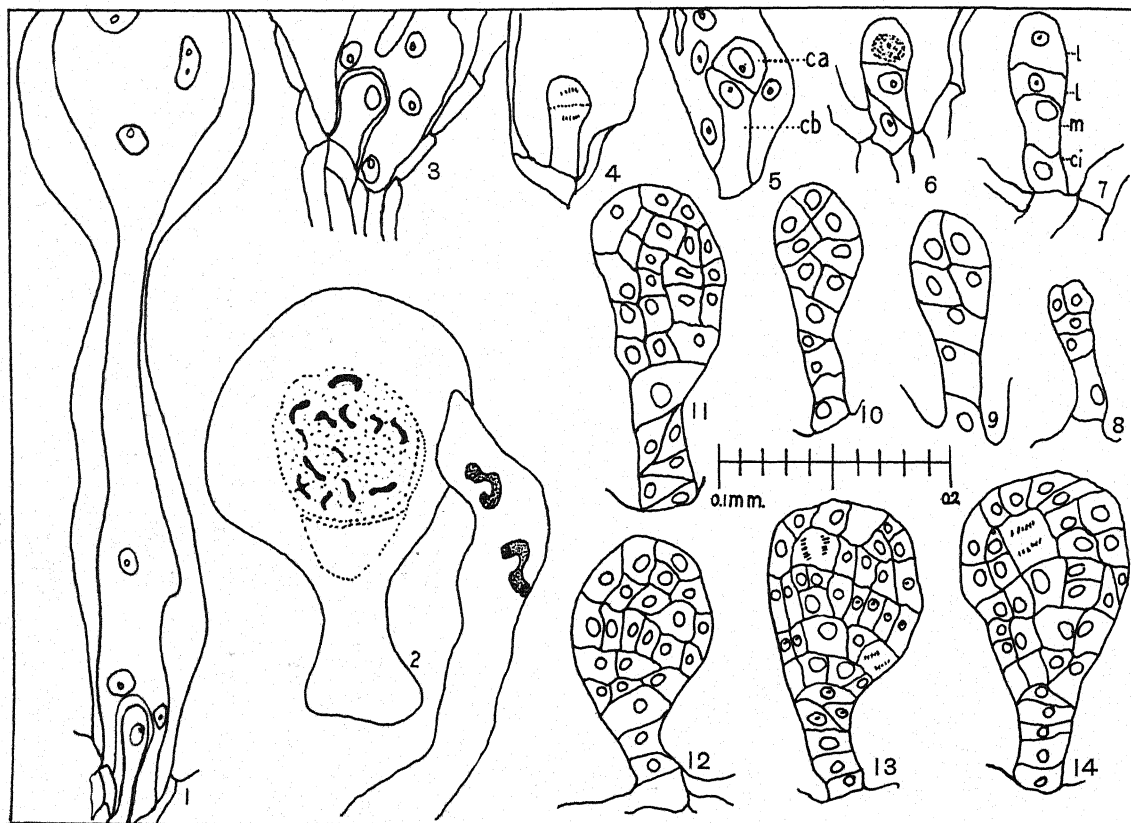


Fig. 1-14. Drawings of mature embryo sacs and proembryos.—Fig. 1. Longitudinal section of an embryo sac of *Phlox Drummondii* Hook. $\times 300$.—Fig. 2. Egg and end of pollen tube at fertilization. $\times 1000$.—Fig. 3. Zygote surrounded by free nucleate endosperm. $\times 300$.—Fig. 4. Zygote at first division, 22 hours. $\times 300$.—Fig. 5. Two-celled filamentous proembryo, 1 day. $\times 300$.—Fig. 6. Three-celled filamentous proembryo, 1+ days. $\times 300$.—Fig. 7. Four-celled filamentous proembryo, 1+ days. $\times 300$.—Fig. 8, 9, 10, and 11. Proembryos showing development from 1 to 3 days. $\times 300$.—Fig. 12, 13, and 14. Proembryos in stages of development during the fourth day. $\times 300$.

cies was chosen for utilitarian reasons: the plant is an annual with a short vegetative period before flowering; it can be grown easily both in the greenhouse and out-of-doors; it has opposite leaves and unilacunar nodes, the former making easier the study by microscopic sections of the symmetrically placed leaves, the latter facilitating the interpretation as each leaf possesses a single leaf trace. Representa-

tive stages from plants grown in the greenhouse were collected and killed in the Allen-Wilson modification of Bouin's fluid.

Material of representative stages was dehydrated in an n-butyl alcohol series (Zirkle, 1930), embedded in rubberized paraffin (56°–58°C. paraffin with 2 per cent paraloid), and cut into serial sections. These

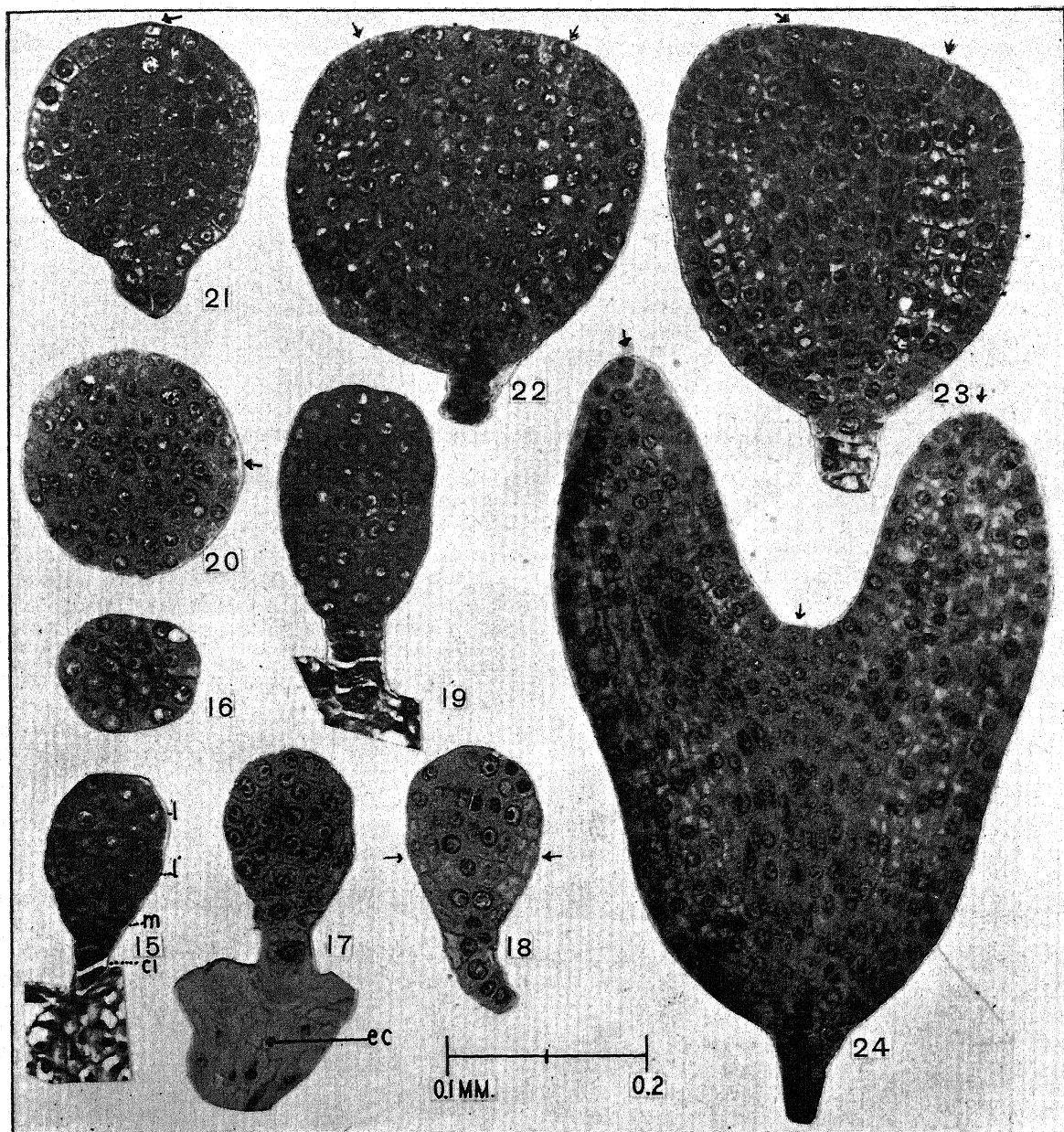


Fig. 15-24. Early stages of proembryo development.—Fig. 15 and 17. Longitudinal sections of a 3-day proembryo. $\times 300$.—Fig. 18. Longitudinal section of a 4-day proembryo. $\times 300$.—Fig. 19. Longitudinal section of a 4+-day proembryo. $\times 300$. Note tiers of cells.—Fig. 20. Transverse section through a 5-day proembryo. $\times 300$.—Fig. 21. Longitudinal section of a 5-day proembryo. $\times 300$. Note tiers of cells.—Fig. 22. Longitudinal section of a 6-day proembryo. $\times 300$. Note tiers are disappearing.—Fig. 23. Longitudinal section of a 6+-day proembryo. $\times 300$. Note appearance of pro-cambial central core and surrounding cylinder.—Fig. 24. Longitudinal section of an 8-day proembryo. $\times 183$.

sections were stained with Heidenhain's iron-alum haematoxylin followed by safranin.

Material of selected stages was placed in lactic acid following killing and kept at 60°C. until the specimens were cleared adequately to show the vascular system. They were further cleared in chloral hydrate and mounted in gum arabic (Massart, 1894).

Under greenhouse conditions, there was a great variation between the times of artificial pollination and fertilization even within an ovary. This variation necessitated an arbitrary assignment of one age to all embryos of a similar morphological stage of development. The youngest age found for a specimen in any certain stage of development was selected as the age for that stage. Thus the time of development assigned was that of the *optimal*, not the *actual*, nor the *average* age for the specimens in that stage. As an example, a five-day embryo, as used in this study, means the most advanced stage seen five days after fertilization, or at least five and one-half days after pollination.

OBSERVATIONS.—The compound pistil of *Phlox Drummondii* is comprised of three carpels. Each of the three loculi contains one or two ovules, attached to an axile placenta, though ordinarily a single ovule matures. The embryo sacs of the family Polemoniaceae, as found by Sundar Rao (1940) in *Polemonium coeruleum*, are designated as *normal* by Maheshwari (1941). The present study on *Phlox Drummondii* supports the conclusions of these men (fig. 1).

Following fertilization which usually occurs from 12 to 36 hours after pollination, the endosperm nucleus begins active division, giving rise to an early free-nucleate endosperm in the embryo sac. The zygote, on the other hand, is a little slower to develop and begins a series of divisions about the time when the endosperm nuclei are being centripetally walled off.

The first three divisions of the zygote are parallel to one another but at right angles to the longitudinal axis of the embryo sac (fig. 4–7). The fourth division occurs in the cell farthest from the point of attachment of the proembryo,³ and divides it longitudinally.

Beyond the filamentous stage there will be considered in order: the *proembryo stage* of two to six days (fig. 28, 29), in which an axially symmetrical mass of cells is supported at the end of a stalk-like suspensor; the *heart-shaped stage* of seven to nine days (fig. 30), in which the respective primordia of the two cotyledons have developed, thus forming a bilaterally symmetrical embryo; the *torpedo stage* of nine to fifteen days (fig. 31–33), in which the cotyledons are partially elongated at the upper end of a recognizable hypocotyl; the *mature embryo* of 30 days (fig. 37), in which the parts of the embryo have reached their final dimensions in the mature seed.

The proembryo stage.—The suspensor supporting the radial proembryo arises from divisions in the lowermost cell of the filamentous four-celled proembryo, called cell *ci* by Souèges (fig. 7). These divisions are transverse to the axis and are usually three in number at this stage, thus giving a suspensor of four cells. The most inferior of these suspensor cells is attached to the inner integument above the micropyle (fig. 15, 17, 19).

Divisions are also initiated in the next most basal cell of the filamentous four-celled proembryo, cell *m* (fig. 7)—termed the *hypophysis* (Hanstein, 1870, and others). The resulting cells contribute to the

³ In this paper, the term *proembryo* is defined after Souèges (1936, p. 48): "On doit entendre plus exactement par proembryon, le corps embryonnaire dérivé de l'ospore, présentant la symétrie simplement axiale de cette cellule et la conservant pendant toute la période qui précède la formation des cotylédons."

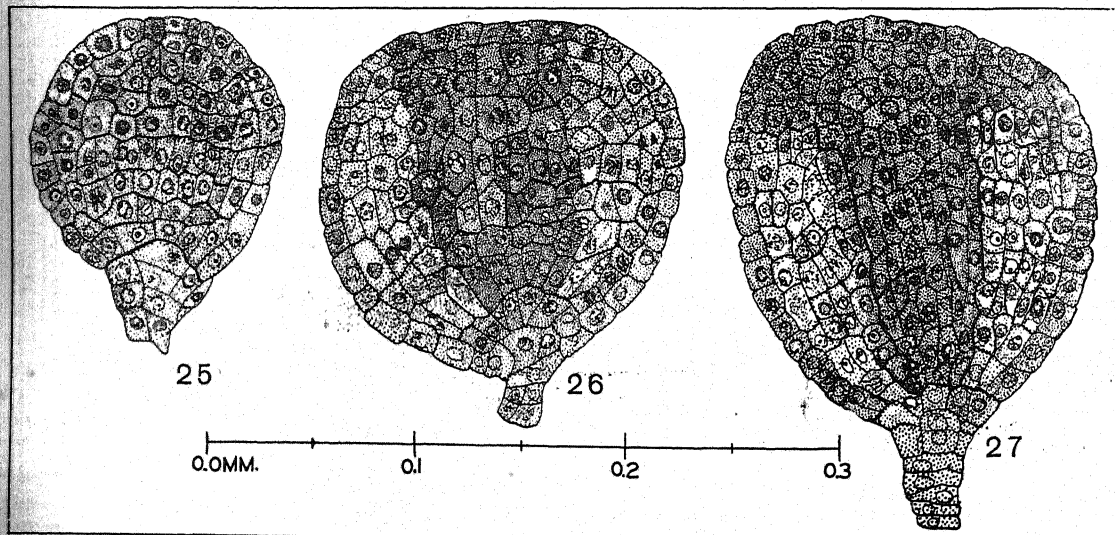


Fig. 25–27. Drawings of proembryos shown in figures 21–23, to emphasize vacuolation, gradual change from homogeneous "tiered" proembryos to heterogeneous proembryos with procambial central core and surrounding cortical cylinder. $\times 300$.

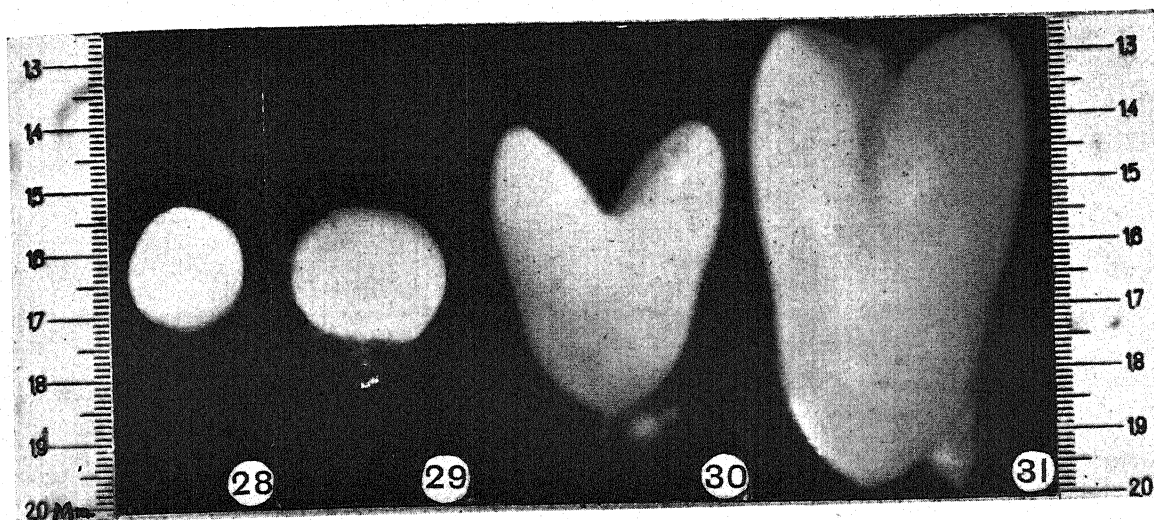


Fig. 28-31. Photographs of proembryos and 8- and 9-day embryos respectively after they have been dissected out of the ovules. $\times 83$.—Fig. 28, 29. Photographs of 5- and 6-day proembryos.—Fig. 30. Photograph of an 8-day embryo in heart-shaped stage.—Fig. 31. Photograph of a 9-day embryo in torpedo-shaped stage. Suspensors broken in dissection.

hypocotyl, the root, the root cap, and the upper part of the suspensor (fig. 15).

The remaining two cells of the filamentous tetrad, l' and l (fig. 7) give rise to the main body of the embryo by many subsequent divisions. There is a certain tendency toward regularity in these divisions which results in a globe-shaped, axillary symmetrical mass of cells (fig. 8-20). By the fourth day (fig. 12-14) periclinal divisions alternate more or less regularly with transverse divisions. In sectional view the resulting proembryo (fig. 21 and 25) appears to be composed of cells arranged in tiers. In the early stages the component cells all tend to have the same general size, shape, and staining capacity (fig. 15-20). Early in the formation of this cell aggregate (16- to 32-celled stage), periclinal divisions generally cease in the peripheral cells. The outer member of each pair of daughter cells (fig. 18, left and right) contributes to the formation of a recognizable outer layer (the *dermatogen* of Hanstein, 1870). In later proembryonic stages, the cells of this outer layer become different in shape and staining capacity from the internal cells (fig. 21), thereby initiating the epidermis of the young embryo. Divisions in this epidermis are characteristically anticlinal except in areas where the cotyledons and the root cap will develop; there, the divisions are both periclinal and anticlinal (fig. 21-27).

The five-day proembryo has a flattened appearance which results from a greater increase in its transverse than in its vertical dimension. A study of cell numbers and cell arrangement (fig. 25, 26) indicates that the periclinal divisions are much more frequent than the transverse divisions, whereas earlier they had been in more or less regular alternation. In longitudinal sections, a fan-shaped area is visible at the base of the proembryonic body. This area, originating from cell m , is composed of larger cells, somewhat triangular in section, and having a

capacity to stain more lightly than those of the internal mass (fig. 21 and 25).

In this five-day proembryo, the arrangement of the transverse rows of cells of the earlier stage has been even more disturbed by a loss of homogeneity of cell size and shape (fig. 22, 26). Instead one can detect within the inner cell mass a *central core* of narrow, elongate, dark-staining cells and a surrounding cylinder of vacuolated light-staining cells (fig. 22, 26). This zonal pattern originates from the differential behavior of the outer, sub-epidermal cells and those in the more axial part of the proembryo. The sub-epidermal cells, which constitute the surrounding cylinder, continue their periclinal division—anticlinal divisions being now infrequent in this region—the daughter cells quickly becoming vacuolated. These cells, therefore, are formed in radial rows. The axial cells, by contrast, divide more frequently in a longitudinal plane and become irregularly elongate. They thus constitute a core of cells, generally longer than wide, which, since they do not become vacuolated, tend to stain densely. Succeeding development of these regions justifies the use of the terms *procambium* for the axial core and *cortex* for the surrounding cylinder in these embryos.

The six-day proembryo has acquired larger dimensions. At the apex there can be seen in longitudinal view a second, broad, fan-shaped area composed of smaller isodiametric, dark-staining meristematic cells in continuity with the central core of elongate, procambial cells (fig. 23, 27). This fan-shaped area is the first indication of the apical meristem of the shoot system. A gradient in size exists from the characteristic cells of this meristem to the axillary elongate, small diametered cells in the region of the central core at lower levels in the proembryo.

Heart-shaped embryo.—About seven or eight days after fertilization, the axial symmetry of the

proembryos is lost with the appearance of the cotyledonary primordia. These primordia appear one on either side of the somewhat flattened top of the proembryo which now becomes somewhat broader than thick. In the presumptive cotyledonary regions, the outer layer shows both anticlinal and periclinal divisions (fig. 22, 23). In the second and third layers, periclinal divisions predominate although anticlinal and oblique divisions may be found (fig. 23, 27). This localized activity produces the cotyledonary primordia within which a pattern of development can soon be recognized. Continued meristematic activity at the apices of the cotyledons results in their apical growth. With the resulting increase in length, there becomes apparent behind each apex a central column of dark-staining procambial cells surrounded by a peripheral zone of vacuolated cells. These developmental changes extend as a wave from the proembryo below, the procambial central core of the proembryo now being continuous upward with the procambial central columns of the cotyledons and the cortex of the proembryo with the peripheral zones surrounding these columns. Thus the procambium now resembles a dichotomously branched system whose arms extend as adaxially grooved and tapering strands into the cotyledons where each strand is

continuous at its apical end with the apical meristem of its cotyledon. In the notch between the procambial strands of the cotyledonary traces, the fan-shaped shoot, or epicotyledonary meristem is still relatively inactive (fig. 23, 27).

Around the embryo, the single-celled external layer is still distinguishable because of the uniformity of cell size and the lighter staining capacity of most of the cells composing it. In this layer at the apex of the cotyledons, there are some periclinal divisions which contribute cells both to it and to the mass of the cotyledons (fig. 24). In the outer layer in the hypophyseal region of the embryo, there is a predominance of periclinal divisions which give rise to the flanking cells of the root cap. The suspensor itself is larger than it was in the former stage, comprising as many as eight cells in length and two cells in diameter (fig. 24, 27).

Torpedo stage.—The cotyledons of a nine- to fifteen-day embryo are oval, dorsiventrally flattened structures at the top of the hypocotyl, the elongate, cylindrical main body of the embryo. This bilaterally symmetrical embryo with dimensions thus changed has been designated the *torpedo stage*.

As in the heart-shaped stage, dark-staining, isodiametric cells are found at the tips of the cotyledons

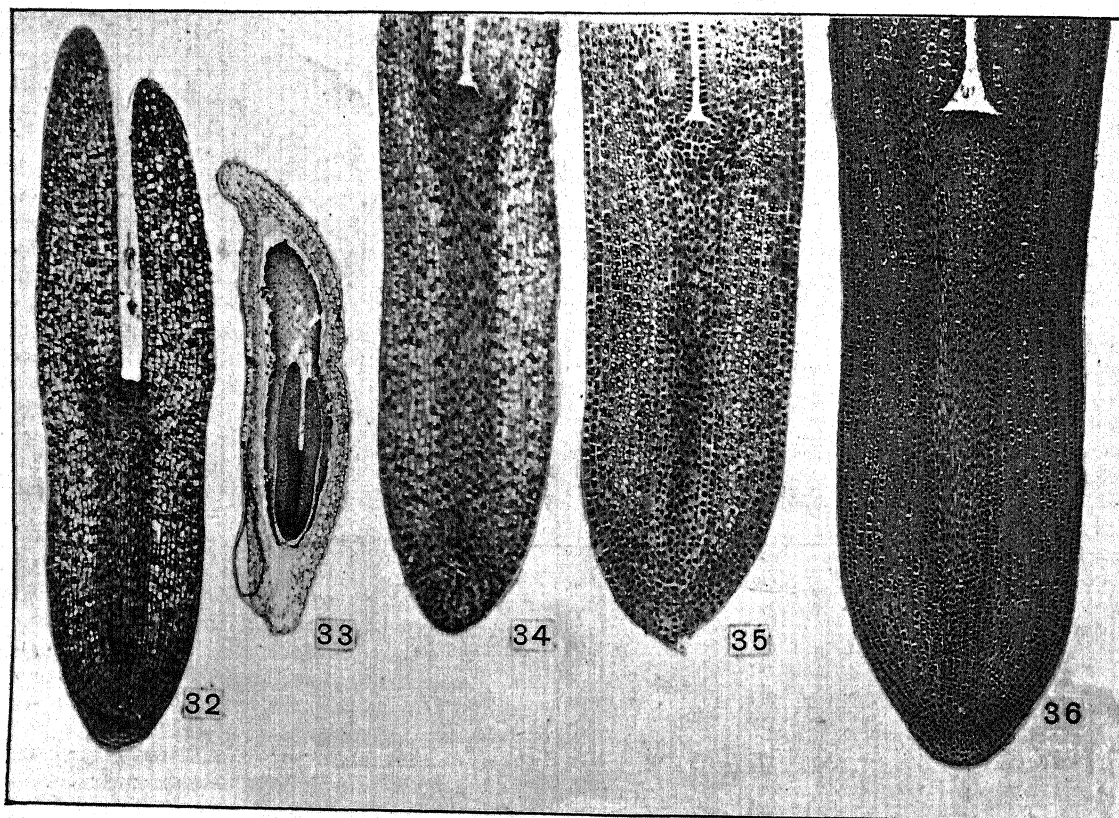


Fig. 32-36. Sections of older stages of embryos; 32-35, torpedo stages; 36, mature stage.—Fig. 32. Longitudinal section of a ± 9 -day embryo. $\times 80$.—Fig. 33. Longitudinal section of a ± 9 -day embryo, enclosed in seed coats. $\times 25$.—Fig. 34-35. Longitudinal section of hypocotyl of an 11- and a 15-day embryo respectively.—Fig. 36. Longitudinal section of hypocotyl of a 30-day embryo. $\times 35$.

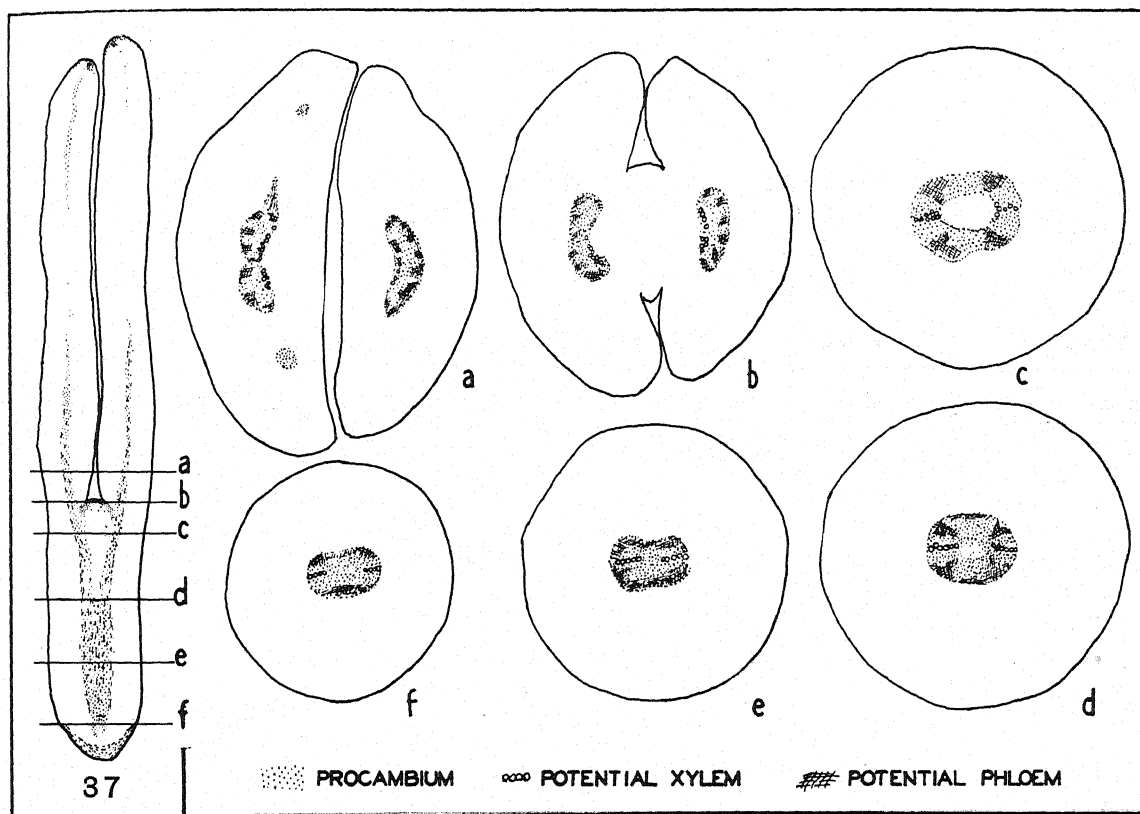


Fig. 37. Drawings of a 30-day embryo to show the early blocking out of regions in the procambial system which in a much older embryo will become protoxylem and protophloem regions.

and in a conical region below the notch between them. In the outer layer covering these regions, periclinal divisions occur along with the more typical anticlinal divisions. In the hypocotyl the central column of elongate, procambial cells has increased in both length and diameter but remains a solid core. The arms of the procambium, extending acropetally into the cotyledons as tapering, adaxially grooved strands, also have gained greater dimensions and have become branched. In embryos of this stage the branches of the strands have never been found to be discontinuous with the main bundle of each cotyledon, suggesting a centrifugal development of the major lateral, cotyledonary bundles.

The root cap which has already been initiated at the basal end of the hypocotyl in the seven- to eight-day embryo (fig. 24), has a dual origin as in many plants. The flanks have been initiated from periclinal divisions in that layer which above is the epidermis of the hypocotyl. The central part of the cap is derived from the distal derivatives of cell division in the hypophyseal region. Just proximal to the root cap, a region of dark-staining cells has also arisen from divisions in the hypophyseal derivatives, thus constituting the meristem for the root. At this stage there may sometimes be found the insignificant remnants of a nonactive suspensor below the root cap (fig. 35).

The mature embryo.—In embryos older than 36 days, the cotyledons apparently enlarge at a more rapid rate than the hypocotyl, for, in the mature embryo, they are longer than the hypocotyl. The root cap is complete at the tip of the meristem of the root axis (fig. 37). No remnant of a suspensor was found at the lower apex of the root cap.

The internal anatomy at this stage attracts special attention as development proceeds beyond the torpedo stage. Below the shoot or epicotyledonary meristem, there is present a recognizable inverted cone of light-staining vacuolated cells, which now separates the shoot meristem from the elongate, dark-staining cells of the top of the procambial core. This light-staining cone is the first indication of a pith in the *Phlox* embryo. As the overall dimensions of the entire embryo become increased during the lapse of time beyond the thirtieth day, so do those of the inverted cone of pith. The cells composing the pith are arranged in linear, vertical rows; the more apical cells in each of these rows are longitudinally shorter than are those more distal to the apex, suggesting their origin from a rib meristem at the base of the apical meristem lying between the cotyledons (fig. 36). Throughout most of the length of the hypocotyl in the mature embryo, the elongate, dark-staining, procambial cells have become a cylinder surrounding the pith; at the base of the hypocotyl, and above the meristem for the root axis, the pro-

cambial cells are still in a central core as in younger stages (fig. 37 e-f).

Toward the end of the development of the mature embryo, the epicotyledonary or shoot meristem becomes more generally active, forming a mound (fig. 26).

Within the procambial system in this maturing embryo faint indications suggest that the location of potential xylem has been determined much before the appearance of the criteria used by Esau for detecting immature protoxylem elements. This early determination of xylem is first evidenced by the tendency for these cells to show shrinkage of protoplasm under the technical procedures employed in this study. It seems legitimate to call these cells *protoxylem mother cells*. Neighboring cells not destined to become xylem do not show this tendency.

Somewhat later one can detect the position of the future phloem by the tendency of cells in that region to become very small in diameter through longitudinal divisions in the procambial cells and through the tendency for the protoplasm of these cells to show slight shrinkage under the same treatments. Such cells of the phloem will be referred to as *protophloem mother cells*.

The spatial relationships of the protoxylem and the protophloem mother cell regions of the procambial strands in the cotyledons, of the procambial

cylinder in the upper hypocotyl, and of the procambial core in the lower hypocotyl are not constant but vary with the level in the embryo. In the upper half of the cotyledons, the protoxylem mother cells are adaxial, the protophloem mother cells abaxial (fig. 37 a). At levels closer to the base of the cotyledons, the aggregations of protoxylem mother cells of each double strand become more medianly situated. By contrast, the aggregations of protophloem cells become more flanking than abaxial (fig. 37 b). In the upper hypocotyl, where the cotyledonary strands become halves of the hypocotyledonary cylinder of procambium, the congery of protoxylem mother cells from each cotyledon assumes a peripheral position in that it is no longer subtended externally by phloem (fig. 37 c). The four regions of protophloem mother cells, however, become two by the joining of adjacent strands, one from each cotyledon; these two resulting protophloem strands come to lie in positions alternating with the two regions of protoxylem mother cells in the cylinder (fig. 37 d). At a lower level in the hypocotyl, where the procambium is a core of cells, the two xylem regions are connected by a row of cells of the now blocked-out metaxylem plate. The phloem mother cells form a small arc at this level on either side of the plate situated between the protoxylem poles.

The transition region in *Phlox* is the pith-contain-

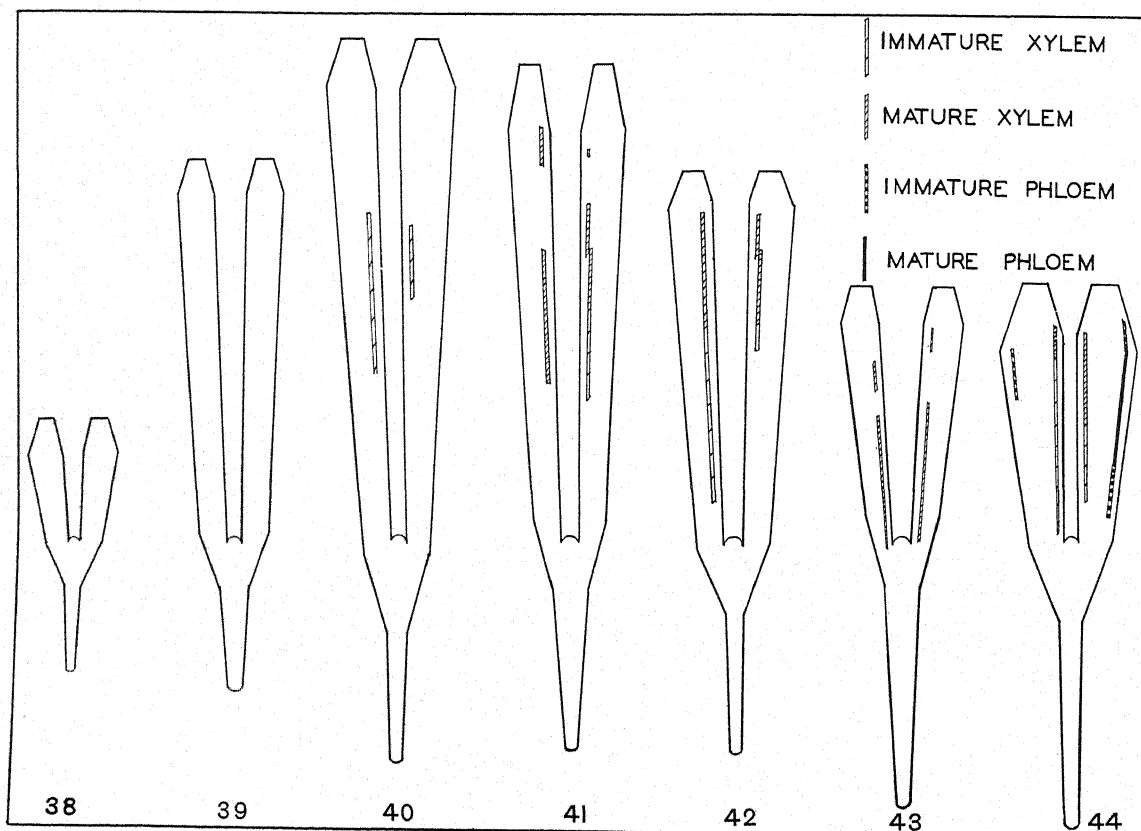


Fig. 38-44.—Fig. 38, 39. Diagrams of 2 embryos in torpedo stage.—Fig. 40-44. Diagrams of different mature embryos showing the maturation of the protoxylem and protophloem, constructed from transverse sections.

ing region of the hypocotyl between the level of the base of the cotyledons and the top of the central core of procambium in the hypocotyl axis. It is clearly evident before any elements of either xylem or phloem are matured. This will be considered in the discussion.

The first of the next series of changes occurs in the cotyledons at about the time of maturing seeds (fig. 40). Certain cells on the adaxial side of the procambial strand may show a helically arranged secondary thickening in the cell wall and a less obvious protoplast; they can therefore be considered as maturing protoxylem. In certain mature, apparently somewhat further developed embryos, there may be cells on the abaxial side of the procambial strand which appear different in that their unstained walls are "nacré" and their nuclei have disappeared; these are identified as protophloem sieve tube elements (fig. 44). Although there may be several independent points of origin for both the protoxylem and protophloem in the cotyledons, the general tendency for further maturation is in both an acropetal and a basipetal direction as the mature embryo develops into a seedling. No sign of protoxylem nor protophloem differentiation has been detected elsewhere in the embryos from any seed of *Phlox Drummondii* investigated.

Discussion.—Since Hanstein's paper in 1870 in which the first descriptions of the development in both monocotyledonous and dicotyledonous embryos appeared, much work upon many species from diverse families has been done (Hegelmaier, 1878; Westermaier, 1876; Treub, 1879; Guignard, 1881; Mottier, 1893; Chauveaud, 1897; van Tieghem, 1897; Schaffner, 1897; Riddle, 1898; and more recently Souèges, 1907-39; etc.). The investigations of these men and others who have contributed to our present knowledge of embryo development have been reviewed at intervals (Guignard, 1881; Ducamp, 1902; Coulter and Chamberlain, 1903; Schnarf, 1929; Souèges, 1936; and Esau, 1943b).

Many attempts have been made, notably by Souèges, to follow cell lineage from the zygote to the later stages of embryo development. Schnarf, following Souèges, has published in his monographic treatment (1929) the more generally used classification of the filamentous stage of embryos according to the planes of the first divisions. The fertilized egg in all four more characteristic types divides transversely, resulting in a two-cell proembryo; the cell of attachment *cb*, and the distal cell *ca* (fig. 5).

In types I and III, cell *cb* does not contribute to the embryo proper, whereas in types II and IV the upper daughter cell from a transverse division in cell *cb* forms the hypophysis which contributes to the formation of the lower embryo and to the upper part of the suspensor. In types I and II, cell *ca* divides longitudinally, whereas in types III and IV it divides transversely.

In 1939, after this present study had been begun, Souèges in a paper on "Embryogénie des Polemoniacees," using *Polemonium coeruleum* as an example,

described the cell lineage for the family as typical of type IV of Schnarf (1929). He found that cell *ci* (fig. 7) gives rise to part of the suspensor; cell *m*, the hypophysis, gives origin to two daughter cells, of which the first initiates the root cap, root and basal part of the hypocotyl, and the second contributes to the suspensor along with cell *ci*; cell *l'* is the precursor to the hypocotyl and the initials of the root; and cell *l* is the forerunner of the epiphysis, the initial related to the origin of cotyledons, hypocotyl proper and epicotyl.

Since *Phlox Drummondii* is a member of the family Polemoniaceae, it is not surprising to note that the planes of the first cell divisions cause this species also to be placed in Schnarf's type IV (fig. 8); thus one would expect to find here the same cell lineage that has been worked out by Souèges and by Bhaduri (1936) for that type. As far as can be observed from killed and sectioned material of *Phlox*, it seems inadvisable to make this generalization, for variations of cell lineage are found even in different specimens of this species. In fact, if one were to cite the case most frequently observed, it would not fit nicely into the cell lineage pattern of type IV, because it appears that cell *m* gives rise to the root primordium as well as to the root cap and the upper part of the suspensor, cell *l'* (fig. 15) to the body of the hypocotyl, and cell *l* to the cotyledons, upper hypocotyl and epicotyl. Hanstein (1870) referred to individual variations, and others since (Hegelmaier, 1878; Guignard, 1881; Chamberlain, 1897; Bhaduri, 1936; Randolph, 1936; Borthwick, 1931; and Nast, 1941) have noted this same lack of exact uniformity of cell lineage and hence warn against generalizations concerning regular sequences of cell divisions, and uniform developmental behavior of the products of such divisions.

Hanstein (1870) was also the first to describe the development of embryos beyond the filamentous stage. In *Capsella Bursa-pastoris* he reports a dermatogen formed in the superior octant of a 20-celled proembryo. This early separation of a peripherally distinct layer has been reported by various workers studying diverse species. In *Phlox*, also, the topographical outside layer becomes histogenically distinct early in the development of the proembryo. Divisions in this layer are predominantly anticlinal although periclinal divisions do occur, especially in the regions where root cap and cotyledons are forming, and occasionally elsewhere. Such periclinal divisions have been reported for other species (Ducamp, 1902). This tunica-like layer thus perpetuates itself by active, anticlinal cell divisions, while the embryo as a whole is still increasing in size by divisions in diverse planes. In the later stages when embryonic growth has gradually become localized in the regions of cotyledon and root cap formation, active cell division in the external layer is likewise localized in those regions. The epidermal layer in older embryos is no longer actively dividing.

The authors favor recognizing the outer layer of the embryo, whether actively dividing or not, as the

epidermis, except in the regions of localized meristems, e.g., the stem apex, the apical and marginal meristems of the leaf. This is in keeping with Linsbauer's (1930) thesis that the epidermis may be considered the topographical boundary of the plant. It is in contrast to Haberlandt's (1914) idea of the epidermis as a tegumentary or dermal layer of physiological import. It eliminates the difficult decision of distinguishing between the stage of active division (the *protoderm* of Haberlandt) and that of the mature epidermis. It also makes it possible to reconcile terminology of the embryo with that of the seedling and the mature plant. (See Parts II and III).

Centrad to the epidermis, the cells in sectioned early proembryos of *Phlox* appear homogeneous and arranged in horizontal rows, suggesting that cell divisions are generally orderly throughout, and that cell enlargement is generally uniform for all cells (fig. 21). Schaffner (1897), Riddle (1898), and Ducamp (1902) speak of these tiers of cells in what Hanstein calls *the inner tissue*.

The loss of this tiering of cells results from a differential behavior between the axial portion of the proembryo and the surrounding peripheral portion. In the former, longitudinal divisions and cell elongation become prominent, thereby destroying the tiering effect. The actual time of the disappearance of homogeneity varies with the species studied (Hanstein, 1870; Ducamp, 1902; Schaffner, 1897; etc.).

Hanstein suggested that this regularity of inner cells gives way to regions called *plerome* and *periblem*. Hanstein (1870), Chamberlain (1897), Ducamp (1902) and others point out that cells of the *plerome* are marked by dense protoplasmic contents. They are characterized by cell elongation and by predominant longitudinal divisions. By contrast, the *periblem* cells become strongly vacuolate and loosely arranged. They show transverse cell divisions rather than cell elongation.

The *plerome* of the above cited papers is clearly the same region that has been called the *central core* of procambium in this paper. The *periblem* is that region referred to as the more lightly staining, *surrounding cylinder* of cortex. The characters used by various authors to distinguish these regions seem applicable to the *Phlox* material. Divisions do occur in the central core but they are, as Hanstein, Ducamp, etc., state, more frequently in a longitudinal than in a transverse plane. It is true that the elongation occurring in the central core is correlated with the overall increase in length by transverse cell division and general cell enlargement in the surrounding cylinder of cortex.

The objection to the use of Hanstein's histogen layers for embryos is the same as has been so well stated by Foster for shoot apices. This objection lies not in his attitude, "which has found increasing support in recent studies, but rather in his attempt to assign specific destinies or prospective values to the various regions of the meristem. . . . In some species the *periblem* and the *plerome* regions are not distinguishable while in other cases where such a de-

marcation might be made, the respective roles of *periblem* and *plerome* do not conform to Hanstein's view" (Foster, 1939, p. 457). In the present paper *plerome* is being replaced by *procambium* and *periblem* by *cortex*, which is in agreement with current practice.

Hanstein (1870) describes the outside layer of the *plerome* as the initial layer for the first procambium in the *Capsella* embryo. Evidence cited in papers by Buchholz and Old (1933) and Nast (1941) suggests that in the plants studied by them it has the same origin. The procambium in *Phlox*, however, seems to arise as an entire central core of dark-staining elongate cells and not from just the outside layer of the core. The pith of *Phlox* appears not to arise from the central cells of the core but as the result of activity of the shoot apical meristem above the core, for at this level there is no pith formed at any time in the life of the plant.

It is significant that the procambium of the cotyledons develops acropetally from the solid procambial core of the lower hypocotyl. Instances have increased in recent years to indicate that this acropetal extension of the procambium into developing leaves is probably characteristic at least for the gymnosperms and angiosperms (Koch, 1891, 1893; Priestley, Scott, and Gillett, 1935; Priestley and Scott, 1937; Crafts, 1943; Boke, 1940, 1941; Cross and Johnson, 1941; Cross, 1942; Esau, 1943a, 1943b; Engard, 1944). So far as the writers know, this investigation of the *Phlox* embryo has provided the first indication of the same developmental pattern for the procambium of cotyledons as is found for the later leaves of the shoot.

At the base of the embryo, above the suspensor, in *Phlox*, the hypophysial tissue contributes to the internal part of the root cap and to the axis of the root. Hanstein (1870) used the term *hypophysis* in a way that could be interpreted as either the cell at the top of the suspensor from which originate the initials of the cortex in the root and the median portion of the cap, or as the cell mass produced by this cell. Souèges (1934) has chosen to distinguish between the "hypophysis" as a cell, and the hypophysial derivatives. He has shown that the hypophysis may give rise to different initials in different genera. In the same study Souèges has considered the "epiphysis" as a cell which appears at the summit of the proembryo and which gives rise to the epiphysial complex. This in turn produces the initials of the epidermis and of the cortex for the upper parts of the embryo. There are also variations in the development of this complex within different genera as is true of the hypophysial derivatives. As far as can be observed in *Phlox* the epiphysis (fig. 15) gives rise to both dermatogen and cortex as described by Souèges.

Many workers (e.g., Guignard, 1881; Riddle, 1898; Billings, 1901) refer to the suspensor in dicotyledons as an absorbing organ supplying nourishment to the rapidly growing embryo. It appears that in *Phlox* the suspensor serves this function and

supplies the young proembryonic stages with nutrients from the integuments which are transported into the suspensor through the elongate cells (fig. 17 ec) at the micropyle (Billings, 1901). In later stages, the heart-shaped and torpedo stages, the suspensor is shriveled and nonactive, suggesting that nutrient material for the embryo must then be absorbed from the endosperm which at this time has become cellular and extensive. The suggested change in nutritional pattern would be in keeping with the findings of Brink and Cooper (1940) on the significance of organized endosperm in the nutrition of the embryo in various species of angiosperms. Observation of this structure in embryos grown in culture should provide an interesting study.

Within the procambium of the cotyledons there is an early determination of protoxylem mother cells and somewhat later an indication of the appearance of protophloem mother cells. Hence the spatial relationship of xylem to phloem is blocked out before any vascular elements become mature. This is not surprising. It is becoming well known that at least the pattern of the whole primary xylem is blocked out in roots before any differentiation of elements is apparent (Esau, 1940, 1943a; Williams, 1940). Wetmore (1943) has reported a corresponding demarcation of primary xylem and of primary phloem as well in the rhizomes of *Lycopodium*. Following Esau, a xylem element was considered mature when the secondary wall was lignified and the cell no longer possessed a nucleus and cytoplasm. A phloem element was called mature when the nucleus had disintegrated completely and the protoplast had become "denatured" and less stainable.

The first evidence of mature vascular elements in *Phlox* appears within the xylem system of mature embryos. At several independent levels in the upper cotyledons, protoxylem elements become mature almost simultaneously. This is in agreement with the condition of early xylem maturation reported in *Keteleeria* by Hutchinson (1917), in *Cedrus* by Buchholz and Old (1933), and also by Hill and de Fraigne (1913).

Only in certain ripe seeds has any evidence been found in *Phlox* embryos that protophloem elements have matured. These reports of earlier protoxylem than protophloem are in contrast to observations reported from other genera. Nast (1941) reports that, in *Juglans*, phloem precedes xylem in the root, the first locus of differentiation. In this species, Nast found that protoxylem was first mature in the cotyledonary traces. In *Lupinus*, Dauphiné and Rivière (1940) report that the first vascular elements to be differentiated are phloem cells which first occur in the hypocotyl-root axis and extend well down to the level of the lateral extensions of the root and above to the cotyledons. However, in the embryos of *Helianthus*, *Ricinus* and *Mirabilis*, these authors report the site of first vascular differentiation to be the cotyledons, and the first elements matured to be the sieve tubes. In flax, Crooks (1933) has found pri-

mary phloem as the first evidence of vascular differentiation in the root at a level 0.4 mm. above the tip of the "plerome" and in a position alternate with the two protoxylem regions which are differentiated later. Some consideration will be given in Part II to the variations in time of appearance and in distribution of the primary vascular elements in embryos and seedlings.

Although much has been done in the matter of interpretation of development of embryos, using killed and sectioned material as a source of information, it now appears that further interpretation may be facilitated by careful observations of embryos grown from zygote to maturity in culture media. To do this it will first be necessary to be able to grow "normal" embryos *in vitro*. The techniques of this process are not yet fully explored but great advances have been made by recent workers in the field, notably van Overbeek and his co-workers. It is hoped that observations of normal *Phlox* embryos grown in culture may soon be made, and experiments can be directed toward some clarification of the factors involved in the differentiation in embryos.

SUMMARY

The development *in situ* of embryos of *Phlox Drummondii* Hook. was studied in successive stages from the first divisions of the zygote to the embryo of the mature seed.

Sections of early proembryos show an axial symmetry and a cellular homogeneity. Those of later proembryos show regions of varying staining capacities: a dark-staining central core of elongate cells, a surrounding region of lightly staining cells, and an enclosing embryonal epidermal layer.

The axially symmetrical proembryos become bilateral, with the appearance of the cotyledons, on or about the seventh day. As the cotyledons expand from the seventh to about the thirtieth day, the above mentioned core of elongate cells becomes extended uninterruptedly into them as characteristic procambial strands, suggesting the procambial nature of the original core. The cortex of the earlier proembryo is also extended as the cotyledons develop.

During this same time, the outer layer of embryonal epidermis divides periclinally in the basal region, forming the root cap. As the cap becomes completed, from the fifteenth to the twentieth day, the suspensor disintegrates and disappears. Interior to the root cap, in the basal region, a hypocotyl meristem appears which, by its activity in the germinating seed, extends the procambium, ground meristem, and epidermis of the hypocotyl and root.

By the activity of a rib meristem of the upper apical hypocotyl meristem, a pith is formed so that the earlier central core of procambium becomes a cylinder. A system of potential xylem becomes recognizable in the procambial system in the late embryonic stages of *Phlox* and becomes extended into the seedling stage as the procambial system is extended. After the position of the potential xylem has been

blocked out in the procambium, the system of potential phloem is established in the remaining procambium farthest removed from the potential xylem.

In the embryo of the mature seed, adaxial cells in the procambial strand of the upper portion of the developing cotyledons have become mature proto-

xylem; meanwhile certain abaxial procambial cells in the basal region of the cotyledons show the characteristics of immature protophloem sieve tubes.

HARVARD BIOLOGICAL LABORATORIES,
HARVARD UNIVERSITY,
CAMBRIDGE, MASSACHUSETTS

LITERATURE CITED

- BHADURI, P. M. 1936. Studies on the embryogeny of the Solanaceae I. Bot. Gaz. 98: 283-295.
- BILLINGS, F. H. 1901. Beiträge zur Kenntniss der Samenentwicklung. Flora 88: 253-318.
- BOKE, N. H. 1940. Histogenesis and morphology of the phyllode in certain species of *Acacia*. Amer. Jour. Bot. 27: 73-90.
- . 1941. Zonation in the shoot apices of *Trichocereus spachianus* and *Opuntia cylindrica*. Amer. Jour. Bot. 28: 656-664.
- BORTHWICK, H. A. 1931. Development of the macrogametophyte and embryo of *Daucus carota*. Bot. Gaz. 92: 23-44.
- BRINK, R. A., AND D. C. COOPER. 1940. Double fertilization and the development of the seed in angiosperms. Bot. Gaz. 102: 1-25.
- BUCHHOLZ, J. T., AND E. M. OLD. 1933. The anatomy of the embryo of *Cedrus* in the dormant stage. Amer. Jour. Bot. 20: 33-44.
- CHAMBERLAIN, C. J. 1897. Contribution to the life history of *Salix*. Bot. Gaz. 23: 147-179.
- CHAUVEAUD, G. 1897. Sur l'évolution des tubes criblés primaires. Compt. Rend. Acad. Sci. Paris 125: 546-547.
- COULTER, J. M., AND C. J. CHAMBERLAIN. 1903. Morphology of the Angiosperms. New York.
- CRAFTS, A. S. 1943. Vascular differentiation in the shoot apex of *Sequoia sempervirens*. Amer. Jour. Bot. 30: 110-121.
- CROOKS, D. M. 1933. Histological and regenerative studies on flax seedlings. Bot. Gaz. 95: 209-239.
- CROSS, G. L. 1942. Structure of the apical meristem and development of the foliage leaves of *Cunninghamia lanceolata*. Amer. Jour. Bot. 29: 288-301.
- , AND T. J. JOHNSON. 1941. Structural features of the shoot apices of diploid and colchicine-induced tetraploid strains of *Vinca rosea* L. Bull. Torrey Bot. Club 68: 618-635.
- DAUPHINÉ, ANDRÉ, ET S. RIVIÈRE. 1940. Sur la présence de tubes criblés dans des embryons de graines non germées. Compt. Rend. Acad. Sci. Paris 211: 359-361.
- DUCAMP, L. 1902. Recherches sur l'embryogénie des *Araliacées*. Ann. Sci. Nat., VIII, Bot. 15: 311-402.
- ENGARD, C. J. 1944. Organogenesis in *Rubus*. Univ. of Hawaii. Res. Pub. 21: 1-234.
- ESAU, K. 1940. Developmental anatomy of the fleshy organ of *Daucus carota*. Hilgardia 13: 175-226.
- . 1942. Vascular differentiation in the vegetative shoot of *Linum*. I. The procambium. Amer. Jour. Bot. 29: 738-747.
- . 1943a. Origin and development of primary vascular tissue in seed plants. Bot. Rev. 9: 125-206.
- . 1943b. Vascular differentiation in the pear root. Hilgardia 15: 299-324.
- FOSTER, A. S. 1939. Problems of structure, growth and evolution in the shoot apex of seed plants. Bot. Rev. 5: 454-470.
- . 1942. Practical plant anatomy. New York.
- GUIGNARD, M. E. 1881. Recherches d'embryogénie végétale comparée. 1^{er} Mémoire: *Légumineuses*. Ann. Sci. Nat., VI, Bot. 11: 5-166.
- HABERLANDT, G. 1914. Physiological plant anatomy. (Eng. Trans.). London.
- HANSTEIN, J. 1868. Die Scheitelzellgruppe im Vegetationspunkt der Phanerogamen. Festschr. Niederrhein. Gesell. Natur. u. Heilkunde: 109-143.
- . 1870. Die Entwicklung des Keimes der Monokotylen und Dikotylen. Bot. Abhandl. aus dem Gebiet der Morph. u. Physiol. 1: 1-112.
- HEGELMAIER, F. 1878. Vergleichende Untersuchungen über Entwicklung dikotyledoner Keime. Stuttgart.
- HILL, T. G., AND E. DE FRATNE. 1913. A consideration of the facts relating to the structure of seedlings. Ann. Bot. 27: 257-272.
- HUTCHINSON, A. H. 1917. Morphology of *Keteleeria fortunei*. Bot. Gaz. 63: 124-135.
- KOCH, L. 1891. Ueber den Bau und das Wachstum der Sprossspitze der Phanerogamen. I. Die Gymnospermen. Jahrb. Wiss. Bot. 22: 491-680.
- . 1893. Die Vegetative Verzweigung der höheren Gewächse. Jahrb. Wiss. Bot. 25: 308-488.
- LINSBAUER, K. 1930. Die Epidermis. Handbuch d. Pflanzenanatomie. IV.
- MAHESHWARI, P. 1941. Recent work on the types of embryo sacs in angiosperms—a critical review. Jour. Indian Bot. Soc. 20: 229-261.
- MASSART, J. 1894. La récapitulation et l'innovation en embryologie végétale. Bull. Soc. Roy. Bot. Belg. 33: 150-247.
- MOTTIER, D. M. 1893. On the embryo sac and embryo of *Senecio aureus* L. Bot. Gaz. 18: 245-253.
- NÄGELI, C. 1858. Das Wachstum des Stammes und der Wurzel bei den Gefäßpflanzen und die Anordnung der Gefäßstränge im Stengel. Beitr. Wiss. Bot. 1: 1-156.
- NAST, C. G. 1941. The embryogeny and seedling morphology of *Juglans regia* L. Lilloa 6: 163-205.
- PRIESTLEY, J. H., AND L. I. SCOTT. 1937. Leaf venation and the leaf trace in the monocotyledon. Proc. Leeds Phil. and Lit. Soc. 3: 159-173.
- , AND E. C. GILLET. 1935. The development of the shoot of *Alstroemeria* and the unit of shoot growth in monocotyledons. Ann. Bot. 49: 161-179.
- RANDOLPH, L. F. 1936. Developmental morphology in the caryopsis of maize. Jour. Agric. Res. 53: 881-916.
- RIDDLE, L. C. 1898. The embryology of *Alyssum*. Bot. Gaz. 26: 314-324.
- RUSSEW, E. 1872. Vergleichende Untersuchungen betreffend die Histologie (Histographie und Histogenie) der vegetativen und sporenbildenden Organe und die Entwicklung der Sporen der Leitbündel-Kryptogamen, mit Berücksichtigung der Histologie der Phanerogamen, ausgehend von der Betrachtung der Marsiliaceen. Acad. Imp. Sci. St. Pétersbourg, Mém. VII, 19: 1-207.
- SACHS, J. 1868. Lehrbuch der Botanik nach dem gegenwärtigen Stand der Wissenschaft. Leipzig.

- SCHAFFNER, J. 1897. Contributions to the life history of *Sagittaria variabilis*. Bot. Gaz. 23:252-273.
- SCHNARF, K. 1929. Embryologie der Angiospermen. Berlin.
- SOUÈGES, R. 1934. L'hypophyse et l'épiphyse; les problèmes d'histogenèse qui leur sont liés. Bull. Soc. Bot. France 81:737-748; 769-778.
- . 1936. La différenciation III.—la différenciation organique. Paris.
- . 1939. Embryogénie des Polemoniacees. Developpement de l'embryon chez le *Polemonium coeruleum* L. Compt. Rend. Acad. Sci. Paris 208:1338-1340.
- SUNDAR RAO, Y. 1940. Male and female gametophytes of *Polemonium coeruleum* Linn. with a discussion of the affinities of the family Polemoniaceae. Proc. Nat. Inst. Sci. India 6:294-361.
- TIEGHEM, PH. VAN. 1897. Morphologie de l'embryon et de la plantule chez les Graminées et les Cypéracées. Ann. Sci. Nat., VIII, Bot. 3:259-309.
- TREUB, M. 1879. Notes sur l'embryogénie de quelques orchidées. Verhand. I. Matuurk. k. Akad. van Wetensch., Amsterdam 19:1-50.
- WESTERMAIER, M. 1876. Die ersten Zelltheilungen im Embryo von *Capsella bursa pastoris* M. Flora 59:483-491, 515-521.
- WETMORE, R. H. 1943. Leaf-stem relationships in the vascular plants. Torrey 43:16-28.
- WILLIAMS, B. C. 1940. Differentiation of vascular tissue in root tips. Abstract. Amer. Jour. Bot. 27:10s.
- ZIRKLE, C. 1930. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science 71:103-104.

NATURAL HYBRIDS BETWEEN ORYZOPSIS HYMENOIDES AND SEVERAL SPECIES OF STIPA¹

B. Lennart Johnson

HITCHCOCK (1935) included *Oryzopsis caduca* Beal in *Oryzopsis Bloomeri*. The type specimen of *O. caduca* was identified by Johnson and Rogler (1943) as a sterile hybrid between *Oryzopsis hymenoides* (Roem. and Schult.) Ricker and *Stipa viridula* Trin. To this natural hybrid the name \times *Stiporyzopsis caduca* (Beal) Johnson and Rogler was applied. It was also pointed out in their study that the type specimen of *Oryzopsis Bloomeri* (Boland.) Ricker probably represents another such hybrid with a different *Stipa* parentage. This led to the question of the proper identity of other specimens identified as *O. Bloomeri* sensu Hitchcock.

An examination of the collections of *O. Bloomeri* from the National Herbarium, the University of California and the University of Minnesota shows that all of the specimens are remarkably similar in general habit (table 1). They are all characterized by an open panicle which is intermediate between that of *O. hymenoides* and the majority of species of *Stipa*. They have a once geniculate awn which is deciduous as in *O. hymenoides* and is intermediate in length (tables 3-5) between the straight awn of *O. hymenoides* and the twice geniculate awn of many species of *Stipa*. In length of pedicels they show a similar intermediacy. In length and shape of glumes they are intermediate between the shorter, ovate glumes of *O. hymenoides* and the longer, lanceolate glumes found in many species of *Stipa*. The lemma is intermediate in length and shape between the broad fusiform lemma of *O. hymenoides* and the narrow fusiform one of the same species

of *Stipa*. The palea and anthers are similarly intermediate in length. *Oryzopsis hymenoides* has ten to twelve beards per anther sac, while in most of the species of *Stipa* investigated the anthers are naked. In the specimens identified as *O. Bloomeri* the anthers have fewer and shorter beards than in *O. hymenoides*. All of these specimens are pollen sterile and produce no seed.

While all of the specimens identified as *O. Bloomeri* show affinity to *O. hymenoides* and the genus *Stipa*, some of them nevertheless differ from each other in certain distinct features in which each resembles a different species of *Stipa* (table 2). They can be readily classified into two groups, those with smooth awns and those with sub-plumose awns. This division corresponds to a similar division in the genus *Stipa* on the basis of smooth or plumose awns. Within each of these main divisions the specimens identified as *O. Bloomeri* can be further subdivided on the basis of characters similar to those used by Hitchcock (1935) in subdivisions of *Stipa*. The specimens are very sporadic in distribution and rare in occurrence. Each one was found to occur where the range of the particular species of *Stipa* which it resembles on morphological characters overlaps the range of *Oryzopsis hymenoides*.

Their common affinity to *O. hymenoides* and their separate affinities to different species of *Stipa* together with their sterility and geographic distribution suggest that the specimens identified as *O. Bloomeri* constitute a group of parallel hybrids having *O. hymenoides* as a common parent.

MATERIALS AND METHODS.—The method used in obtaining data from herbarium specimens of the hybrids, their parental species of *Stipa* and *O. hymenoides* has been described elsewhere (Johnson and Rogler, 1943). The data presented in tables 1 to 5 are based on five spikelets from each specimen

¹ Received for publication July 5, 1945.

The writer is indebted to Dr. C. O. Rosendahl and Dr. F. K. Butters of the Botany Department, University of Minnesota, for advice and help in the investigation of this problem, also to Dr. C. R. Burnham of the Division of Agronomy and Plant Genetics, University of Minnesota, and to Mr. G. A. Rogler, Northern Great Plains Field Station, Mandan, North Dakota, who suggested the original problem involving \times *Stiporyzopsis caduca*.

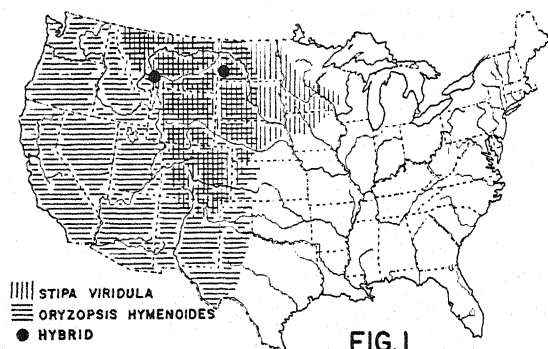


FIG. 1

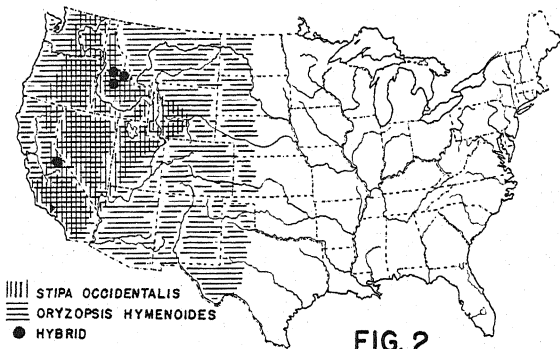


FIG. 2

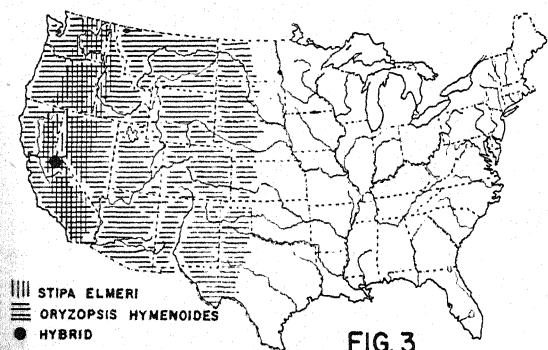


FIG. 3

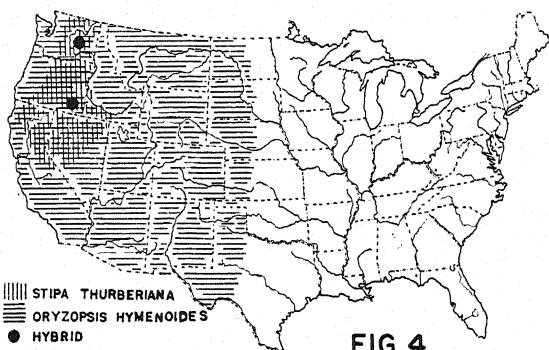


FIG. 4

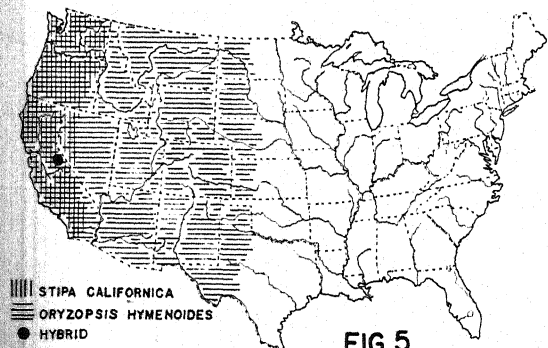


FIG. 5

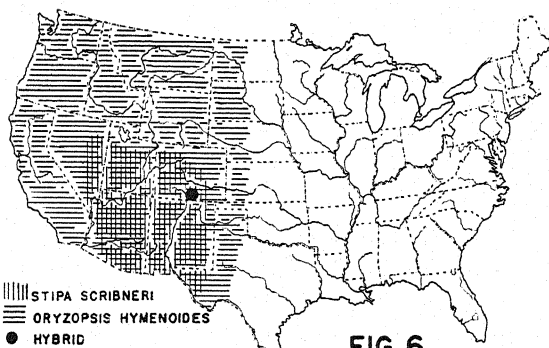


FIG. 6

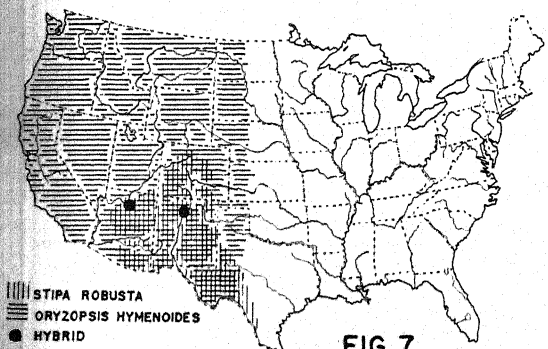


FIG. 7

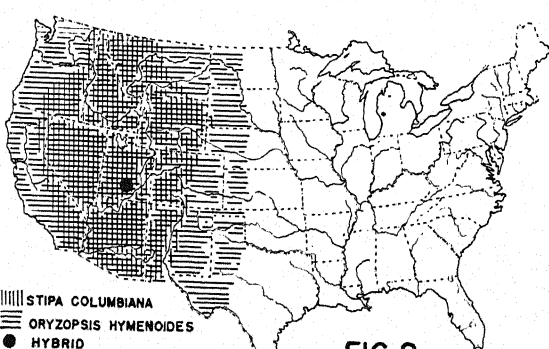


FIG. 8

Fig. 1-8. Maps of geographic distribution of \times *Stiporyzopsis* hybrids and their parental species.

unless otherwise indicated. No attempt was made to sample the entire range of the species of *Stipa*. Instead, if available, a specimen was selected from the state or precise locality in which its hybrid was found. The illustrations were traced from photographs made to give a magnification of $20\times$ except

for *O. hymenoides* in which case camera lucida drawings were made at the same magnification.

Key to \times Stiporyzopsis Hybrids

1. Awn sub-plumose below the bend
2. Sheaths pubescent

(*Oryzopsis hymenoides* \times *Stipa Elmeri*)

2. Sheaths glabrous or slightly scaberulous, sometimes with a few hairs at the throat
3. Hairs of equal length all over the lemma, less than 2 mm. long
4. Ligule less than 2 mm. long, sheaths shiny glabrous
 \times *Stiporyzopsis Bloomeri* (*O. hymenoides* \times *S. occidentalis*)
4. Ligule more than 2 mm. long, lower sheaths scaberulous
 $(O. hymenoides \times S. Thurberiana)$
3. Hairs on upper part of lemma longer than those below, more than 2 mm. long
 $(O. hymenoides \times S. californica)$
1. Awn scabrous or nearly glabrous
5. Hairs of the lemma more than 2.5 mm. long
 $(O. hymenoides \times S. Scribneri)$
5. Hairs of the lemma less than 2.5 mm. long
6. Some of the sheaths villous at the throat or collar
7. Hairs of the lemma about 1 mm. long, anthers mostly abortive less than 1 mm. long
 \times *Stiporyzopsis caduca* (*O. hymenoides* \times *S. viridula*)
7. Hairs of the lemma about 2 mm. long, anthers not abortive, more than 2 mm. long
 $(O. hymenoides \times S. robusta)$
6. Sheaths glabrous at the throat and collar
 $(O. hymenoides \times S. columbiana)$
1. \times *STIPORYZOPSIS CADUCA* (Beal) Johnson and Rogler (*Oryzopsis hymenoides* \times *Stipa viridula*). Amer. Jour. Bot. 30: 49-56. 1943.
Oryzopsis caduca Beal, Bot. Gaz. 16: 111.

TABLE 1. Morphological characters of parental species and of seven hybrids previously identified as *Oryzopsis Bloomeri*.

	<i>Oryzopsis hymenoides</i>	Seven parental species of <i>Stipa</i>	Seven hybrids
Panicle	Diffuse	Narrow	Open
Glumes			
Shape	Ovate	Lanceolate	Narrow ovate
Number of nerves			
First glume	3 (5) ^a	3-5	3 (5)
Second glume	(3) 5 (7)	3, 5	3, 5
Lemma			
Shape	Broad fusiform	Narrow fusiform	Fusiform
Anther beards per sac	(4) 10-12 (18)	0	5-12
Awn			
Persistence	Deciduous	Persistent	Deciduous
Geniculation	Straight	Twice	Once
Pollen	Normal	Normal	Sterile

^a The numbers in parenthesis represent a few exceptional cases.

The data for *Oryzopsis hymenoides* are taken from Johnson and Rogler, Amer. Jour. Bot. (1943).

TABLE 2. Morphological characters of parental species and hybrids.

	No. of nerves in lemma	Indument of awn	Throat of sheath
<i>Oryzopsis hymenoides</i>	(3) 5 ^a	Scabrous	Glabrous
<i>Stipa occidentalis</i>	(3) 5 (8)	Plumose	Glabrous
\times <i>Stiporyzopsis Bloomeri</i> (<i>O. hymenoides</i> \times <i>S. occidentalis</i>) including type.....	5	Sub-plumose	Glabrous
<i>S. Elmeri</i>	5	Plumose	Pubescent
$(O. hymenoides \times S. Elmeri)$	5	Sub-plumose	Pubescent
<i>S. Thurberiana</i>	5	Plumose	Glab. or scaberulous
$(O. hymenoides \times S. thurberiana)$	5	Sub-plumose	Glab. or scaberulous
<i>S. californica</i>	5 (6)	Plumose	Glab. or with a few hairs
$(O. hymenoides \times S. californica)$	5 (7)	Sub-plumose	Glab. or with a few hairs
<i>S. Scribneri</i>	5	Scabrous	Glab. or with a few hairs
$(O. hymenoides \times S. Scribneri)$	5	Scabrous	Sparsely villous
<i>S. robusta</i>	5-6 (7)	Scabrous	Densely villous
$(O. hymenoides \times S. robusta)$	5 (7)	Scabrous	Villous
<i>S. columbiana</i>	5 (7)	Scabrous	Glabrous
$(O. hymenoides \times S. columbiana)$	5	Scabrous	Glabrous

^a The numbers in parenthesis represent a few exceptional cases.

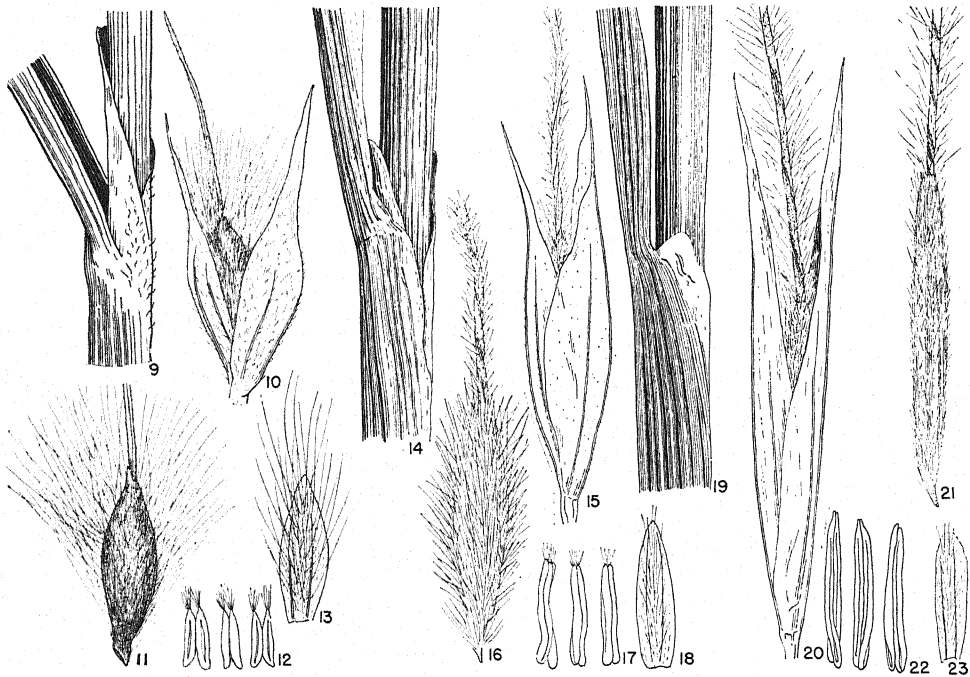


Fig. 9-23. Floral and vegetative parts.—Fig. 9-13. *Oryzopsis hymenoides*.—Fig. 9. Ligule and throat of sheath.—Fig. 10. Spikelet.—Fig. 11. Lemma.—Fig. 12. Anthers.—Fig. 13. Palea.—Fig. 14-18. \times *Stiporyzopsis Bloomeri* (*Oryzopsis hymenoides* \times *Stipa occidentalis*) type.—Fig. 14. Ligule and throat of sheath.—Fig. 15. Spikelet.—Fig. 16. Lemma.—Fig. 17. Anthers.—Fig. 18. Palea.—Fig. 19-23. *Stipa occidentalis*.—Fig. 19. Throat of sheath.—Fig. 20. Spikelet.—Fig. 21. Lemma.—Fig. 22. Anthers.—Fig. 23. Palea. All $\times 8$. (Figures 9-13 from Johnson and Rogler, Amer. Jour. Bot.)

1890. Belt Mountains, Mont., Scribner.

A discussion of cytological and morphological evidence of the parentage of this hybrid is given by Johnson and Rogler (loc. cit.) and need not be repeated here. The geographic ranges of the parent species and the hybrid are illustrated in figure 1.

2. \times ***Stiporyzopsis Bloomeri*** (Bolander.), comb. nov. (*Oryzopsis hymenoides* \times *Stipa occidentalis*).

Stipa Bloomeri Bolander, Proc. Calif. Acad. Sci. 4: 168. 1872. Bloody Canyon, near Mono Lake, Calif., Bolander 6116.

Oryzopsis Bloomeri (Bolander.) Ricker ex Piper, Contrib. U. S. Natl. Herb. 11-109, 1906.

Notwithstanding the close resemblance between the types of \times *Stiporyzopsis caduca* and \times *Stiporyzopsis Bloomeri* on the majority of morphological characters as well as pollen sterility, certain distinct differences exist which preclude the possibility of a common *Stipa* parentage. \times *Stiporyzopsis caduca* resembles *Stipa viridula* Trin., its *Stipa* parent, in having a glabrous awn, a sparsely villous throat of the sheath, seven nerves in the lemma and dimorphous anthers while \times *Stiporyzopsis Bloomeri* has an awn which is sub-plumose below the bend, a glabrous throat of the sheath, five nerves in the lemma (table 2) and equal anthers (table 3). Also in the type of \times *Stiporyzopsis caduca* the ligule is

3 mm. long while in the type of \times *Stiporyzopsis Bloomeri* (table 3) it is 1.6 mm. long.

The features in which the two type specimens differ indicate that the *Stipa* parent involved in \times *Stiporyzopsis Bloomeri* has a plumose or sub-plumose awn, a glabrous throat of the sheath, a lemma with commonly not more than five nerves, equal anthers and a shorter ligule than *Stipa viridula*.

A search of the collections in the herbarium at the University of Minnesota showed that specimens identified as *O. Bloomeri*, *O. hymenoides* and *S. occidentalis* Thurb. had been collected by W. and D. Eggler in craters of the Moon National Monument, Idaho. The herbarium labels give the same date, locality and elevation for all of these specimens. Additional specimens are included from the same locality with slightly different elevations cited, but no other species of *Stipa* or *Oryzopsis* are represented in their collection from this station.

An examination of the material identified as *O. Bloomeri* shows it to be pollen sterile and to have set no seed. In morphological characters (tables 2 and 3) it is similar to the type specimen. Both this material and the type (fig. 14-18) are intermediate between *S. occidentalis* (fig. 19-23) (tables 2 and 3) and *O. hymenoides* (fig. 9-13) (tables 2 and 3) in the majority of characters. They evidently are hybrids between those two species. *Stipa occidentalis* with a plumose awn, five nerves in the lemma,

TABLE 3. Mean lengths (with S.D.) in mm. of floral and vegetative parts of parental species, hybrid and type specimen.

	<i>Oryzopsis</i> <i>hymenoides</i>	<i>Stipa</i> <i>occidentalis</i>	(<i>O. hymenoides</i> × <i>S. occidentalis</i>)	<i>Oryzopsis</i> <i>Bloomeri</i> (Type)
Pedicels				
Longer	14.7 ± 4.4	9.5 ± 3.4	11.6 ± 1.7	10.1 ± 1.9
Shorter	11.0 ± 3.8	2.3 ± 0.8	7.2 ± 1.8	4.8 ± 1.4
Glumes				
First	6.3 ± 1.6	12.3 ± 0.4	8.0 ± 0.4	7.9 ± 0.4
Second	5.6 ± 0.8	11.8 ± 0.5	7.2 ± 0.4	7.3 ± 0.4
Lemma	3.3 ± 0.3	6.7 ± 0.4	4.4 ± 0.2	4.5 ^a
Hairs of the lemma	2.9 ± 0.4	0.6 ± 0.2	1.8 ± 0.1	1.2 ± 0.1
Palea	2.8 ± 0.3	2.9 ± 0.3	2.9 ± 0.1	2.6 ^a
Anthers				
Longest	1.3 ± 0.3	3.7 ± 0.6	2.3 ± 0.1	2.0 ^a
Next to longest	1.2 ± 0.2	3.5 ± 0.5	2.3 ± 0.1	2.0 ^a
Anther beards	0.4 ± 0.1	none	0.4 ± 0.0	0.4 ^a
Awn	4.5 ± 1.1	34.2 ± 4.7	14.4 ± 1.1	13.4 ± 0.3
Ligule	4.7 ± 1.0 ^b	0.3 ± 0.1	1.3 ± 0.5	1.6 ^a
Number of measurements.	60	10	10	5

^a Based on a single measurement.^b Based on 43 measurements.

The data for *Oryzopsis hymenoides* and the type of *Oryzopsis Bloomeri* are taken from Johnson and Rogler, Amer. Jour. Bot. (1943).

a glabrous throat of the sheath, an extremely short ligule about .3 mm. long, and equal anthers showing no abortion supplies the characters in which this hybrid differs from × *Stiporyzopsis caduca*. The name of the hybrid between *S. occidentalis* and *O. hymenoides* therefore should be × *Stiporyzopsis Bloomeri* (Boland.).

Four collections of this hybrid have been exam-

ined, including the type collected in Bloody Canyon, California, and three made by different collectors at Craters of the Moon, Idaho. The ranges of *Stipa occidentalis* and *O. hymenoides* (fig. 2) overlap in both of these localities. Hitchcock (1925) cites specimens of *S. occidentalis* collected by Bolander along Mariposa Trail and Yosemite Trail, California.

TABLE 4. Mean lengths (with S.D.) in mm. of floral and vegetative parts of parental species of *Stipa* and the hybrids between these species and *Oryzopsis hymenoides*.

	<i>Stipa</i> <i>Elmeri</i>	(<i>O. hymenoides</i> × <i>S. Elmeri</i>)	<i>Stipa</i> <i>Thurberiana</i>	(<i>O. hymenoides</i> × <i>S. Thurberiana</i>)	<i>Stipa</i> <i>californica</i>	(<i>O. hymenoides</i> × <i>S. californica</i>)
Pedicels						
Longer	5.5 ± 0.7	12.3 ± 1.6	8.5 ± 3.3	10.6 ± 2.3	6.5 ± 1.5	11.6 ± 1.6
Shorter	1.6 ± 0.2	6.4 ± 0.6	2.9 ± 1.5	6.2 ± 1.2	2.1 ± 0.6	6.5 ± 2.2
Glumes						
First	12.4 ± 0.8	9.2 ± 0.3	11.7 ± 1.0	7.7 ± 0.5	10.8 ± 1.1	10.2 ± 0.7
Second	12.1 ± 0.6	8.3 ± 0.3	10.0 ± 0.8	7.1 ± 0.5	10.3 ± 1.1	8.6 ± 0.7
Lemma	7.2 ± 0.2	5.5 ± 0.2	7.4 ± 0.5	4.7 ± 0.2	6.6 ± 0.8	5.5 ± 0.4
Hairs of the lemma	0.6 ± 0.1	1.4 ± 0.1	0.7 ± 0.1	1.6 ± 0.0	1.8 ± 0.3	2.3 ± 0.1
Palea	2.8 ± 0.2	3.4 ± 0.1	5.3 ± 0.3	3.1 ± 0.2	2.7 ± 0.2	4.1 ± 0.3
Anthers						
Longest	3.7 ± 0.2	2.7 ± 0.1	4.4 ± 0.3 ^a	2.3 ± 0.3	3.1 ± 0.4	2.7 ± 0.2
Next to longest	3.6 ± 0.2	2.6 ± 0.1	4.2 ± 0.4 ^a	2.2 ± 0.3	3.0 ± 0.4	2.6 ± 0.2
Anther beards	none	0.5 ± 0.0	none	0.4 ± 0.0	none	0.5 ± 0.1
Awn	35.2 ± 1.8	15.1 ± 1.0	34.3 ± 4.6	15.7 ± 1.1	29.4 ± 4.3	13.8 ± 0.8
Ligule	0.3 ± 0.1	0.7 ± 0.1 ^b	5.1 ± 1.2	2.3 ± 0.5 ^c	0.3 ± 0.1 ^d	1.7 ± 0.3 ^b
No. of measurements	5	5	10	10	10	5

^a Based on six measurements. ^b Based on three measurements. ^c Based on nine measurements. ^d Based on five measurements.

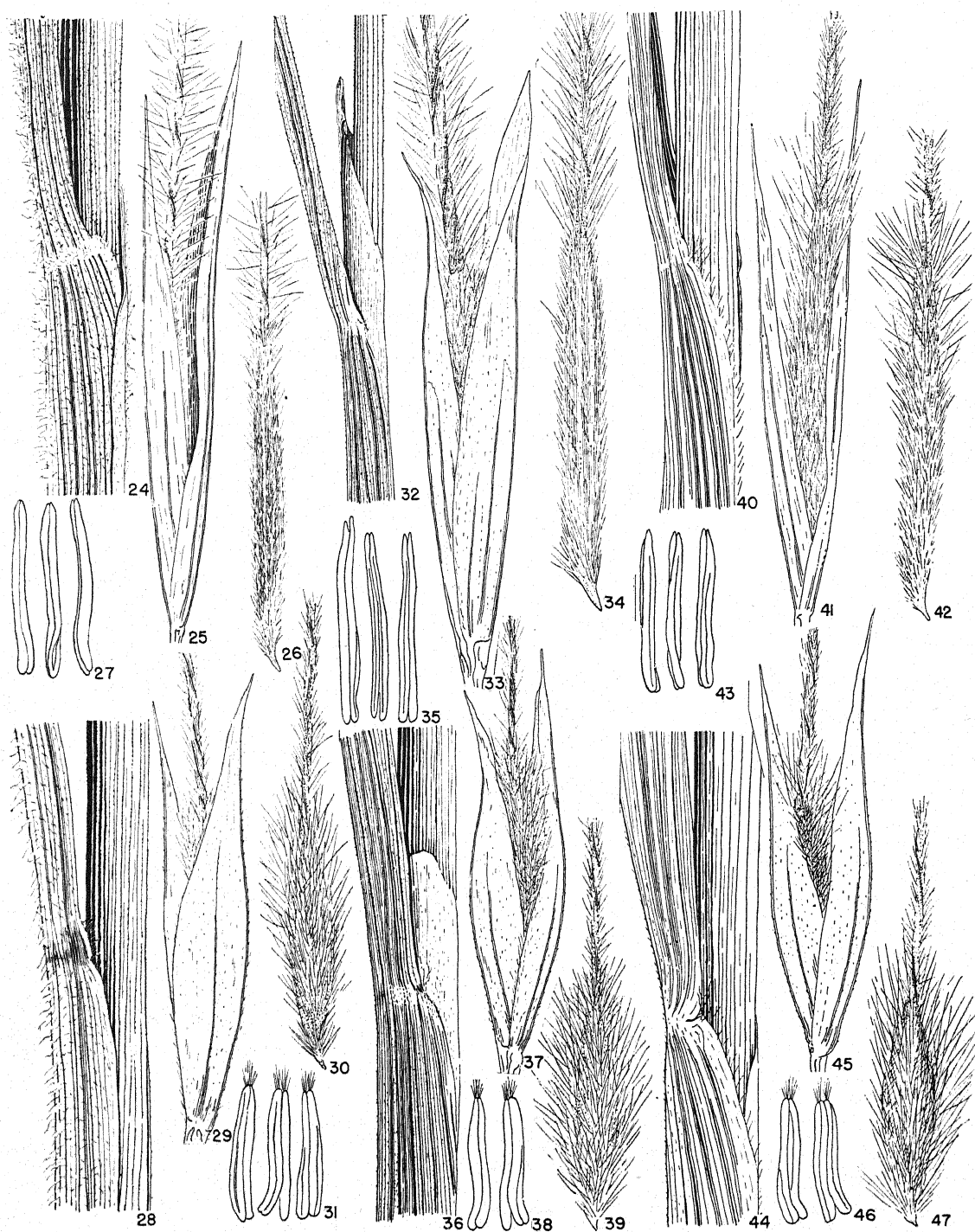


Fig. 24-47. Floral and vegetative parts.—Fig. 24-27. *Stipa Elmeri*.—Fig. 24. Throat of sheath.—Fig. 25. Spikelet.—Fig. 26. Lemma.—Fig. 27. Anthers.—Fig. 28-31. (*O. hymenoides* x *S. Elmeri*) type.—Fig. 28. Throat of sheath.—Fig. 29. Spikelet.—Fig. 30. Lemma.—Fig. 31. Anthers.—Fig. 32-35. *Stipa Thurberiana*.—Fig. 32. Ligule and throat of sheath.—Fig. 33. Spikelet.—Fig. 34. Lemma.—Fig. 35. Anthers.—Fig. 36-39. (*O. hymenoides* x *S. Thurberiana*) type.—Fig. 36. Ligule and throat of sheath.—Fig. 37. Spikelet.—Fig. 38. Two anthers.—Fig. 39. Lemma.—Fig. 40-43. *Stipa californica*.—Fig. 40. Throat of sheath.—Fig. 41. Spikelet.—Fig. 42. Lemma.—Fig. 43. Anthers.—Fig. 44-47. (*O. hymenoides* x *S. californica*) type.—Fig. 44. Throat of sheath.—Fig. 45. Spikelet.—Fig. 46. Two anthers.—Fig. 47. Lemma. All $\times 8$.

TABLE 5. Mean lengths (with S.D.) in mm. of floral and vegetative parts of parental species of *Stipa* and the hybrids between these species and *Oryzopsis hymenoides*.

	<i>Stipa</i> <i>Scribneri</i>	(<i>O. hymenoides</i> × <i>S. Scribneri</i>)	<i>Stipa</i> <i>robusta</i>	(<i>O. hymenoides</i> × <i>S. robusta</i>)	<i>Stipa</i> <i>columbiana</i>	(<i>O. hymenoides</i> × <i>S. columbiana</i>)
Pedicels						
Longest	7.3 ± 1.1	10.4 ± 1.4	5.5 ± 1.3	10.9 ± 1.2	5.7 ± 1.2	8.4 ± 0.8
Shorter	2.2 ± 0.4	6.2 ± 1.0	2.4 ± 0.8	6.8 ± 1.6	2.0 ± 0.7	5.3 ± 0.4
Glumes						
First	13.2 ± 1.4	8.7 ± 0.7	9.6 ± 0.7	8.5 ± 0.7	7.2 ± 1.3	8.0 ± 0.3
Second	10.3 ± 0.8	7.4 ± 0.4	8.6 ± 1.1	7.1 ± 0.8	7.3 ± 1.3	7.3 ± 0.3
Lemma	7.3 ± 0.6	4.5 ± 0.2	5.8 ± 0.5	4.9 ± 0.5	5.2 ± 0.6	4.5 ± 0.1
Hairs of the lemma	2.2 ± 0.7	3.0 ± 0.2	1.7 ± 0.1	2.0 ± 0.2	0.7 ± 0.1	2.0 ± 0.1
Palea	3.1 ± 0.5	3.3 ± 0.1	4.0 ± 0.3	3.4 ± 0.5	2.3 ± 0.2	3.2 ± 0.1
Anthers						
Longest	4.0 ± 0.4	2.3 ± 0.2	3.8 ± 0.4	2.4 ± 0.3	2.9 ± 0.6	2.0 ± 0.3
Next to longest	4.0 ± 0.4	2.2 ± 0.2	3.6 ± 0.4	2.3 ± 0.3	2.7 ± 0.5	2.0 ± 0.4
Anther beards	none	0.4 ± 0.0	none	0.4 ± 0.0	none	0.4 ± 0.0
Awn	19.4 ± 2.1	11.3 ± 1.1	23.2 ± 2.6	17.3 ± 1.2	19.3 ± 4.4	18.0 ± 1.2
Ligule	0.4 ± 0.2 ^a	1.9 ± 0.2 ^b	1.9 ± 0.4 ^c	2.6 ± 0.6	0.1 ± 0.0 ^d	2.0 ± 0.6
No. of measurements	10	5	10	10	10	5

^a Based on nine measurements. ^b Based on four measurements. ^c Based on seven measurements. ^d Based on eight measurements.

The following herbarium specimens² were used in compiling the data for tables 1, 2, and 3:

Stipa occidentalis. California: Mt. Goddard, Hall and Chandler 708 (UM). Idaho: Craters of the Moon, Egger 73 (UM).

× *Stiporyzopsis Bloomeri* (*O. hymenoides* × *S. occidentalis*). California: Bloody Canyon, 1871, Bolander (CAS) Type. Idaho: Craters of the Moon, Egger 147 (UM); Chase 5832 (US); Christ 1931 (UI).

3. (*Oryzopsis hymenoides* × *Stipa Elmeri*). hybr. nov., aristis sub-plumosis, vaginis pubescentibus, ligulis minoribus quam 2 mm. longis, villis lemmatis minoribus quam 2 mm. longis.

In size and habit of plant as well as in size of floral parts (fig. 28-31) (table 4) and in other morphological characters (table 2) this hybrid is very similar to × *Stiporyzopsis Bloomeri*. The glumes have the same shape and nervation. The lemma is slightly longer but is five-nerved and has hairs of about the same length as in × *Stiporyzopsis Bloomeri*. The awn is sub-plumose in both cases and the ligule is short. The sheath of the hybrid involving *Stipa Elmeri* Piper and Brodie, however, is pubescent as in the *Stipa* parent in contrast to the glabrous sheath of × *Stiporyzopsis Bloomeri*. A comparison of *Stipa Elmeri* (fig. 24-27) (tables 2 and 4) with *Stipa occidentalis* (fig. 19-23) (tables 2 and 3) shows that the pubescence of the

sheath is also the only feature in which the *Stipa* parents differ noticeably.

Only one specimen of this hybrid was examined. It had set no seed and showed complete pollen sterility. It was collected in Mono County, California, at the edge of the range of *S. Elmeri* (fig. 3), and within the range of *O. hymenoides*.

The following specimens provided the data for *Stipa Elmeri* and (*O. hymenoides* × *S. Elmeri*) included in tables 1, 2 and 4:

Stipa Elmeri. Washington: Okanogan County, Elmer 487 (UM).

(*O. hymenoides* × *S. Elmeri*). California: Mono County, Yates 6276 (UC) Type.

4. (*Oryzopsis hymenoides* × *Stipa Thurberiana*). hybr. nov., aristis sub-plumosis, vaginis interdem scaberulis, ligulis pluribus quam 2 mm. longis, villis lemmatis minoribus quam 2 mm. longis.

In habit of plant and size of floral parts (fig. 36-39) (table 4), as well as in other details of morphology of the floral parts (table 2), this hybrid also is hardly distinguishable from × *Stiporyzopsis Bloomeri*. However, the ligule is considerably longer, being about 2.3 mm. long while in × *Stiporyzopsis Bloomeri* it is 1.3 mm. long. This suggests the greater length of ligule in *Stipa Thurberiana* (fig. 32-35) (tables 2 and 4). In that species the ligule is 5.1 mm. long. Thus the mean ligule length in both *S. Thurberiana* and *O. hymenoides* exceeds that of their assumed hybrid. This hybrid, however, also resembles *S. Thurberiana* in the frequently scaberulous lower sheaths and the puberulent lower nodes of the culms. The hybrid is sterile both as to pollen and seed.

² The herbaria from which specimens were loaned are designated by letters as follows: University of California, Berkeley, (UC); United States National Museum, (US); Missouri Botanical Garden, (MBG); University of Idaho, Moscow, (UI); University of Minnesota, (UM); California Academy of Science, (CAS).

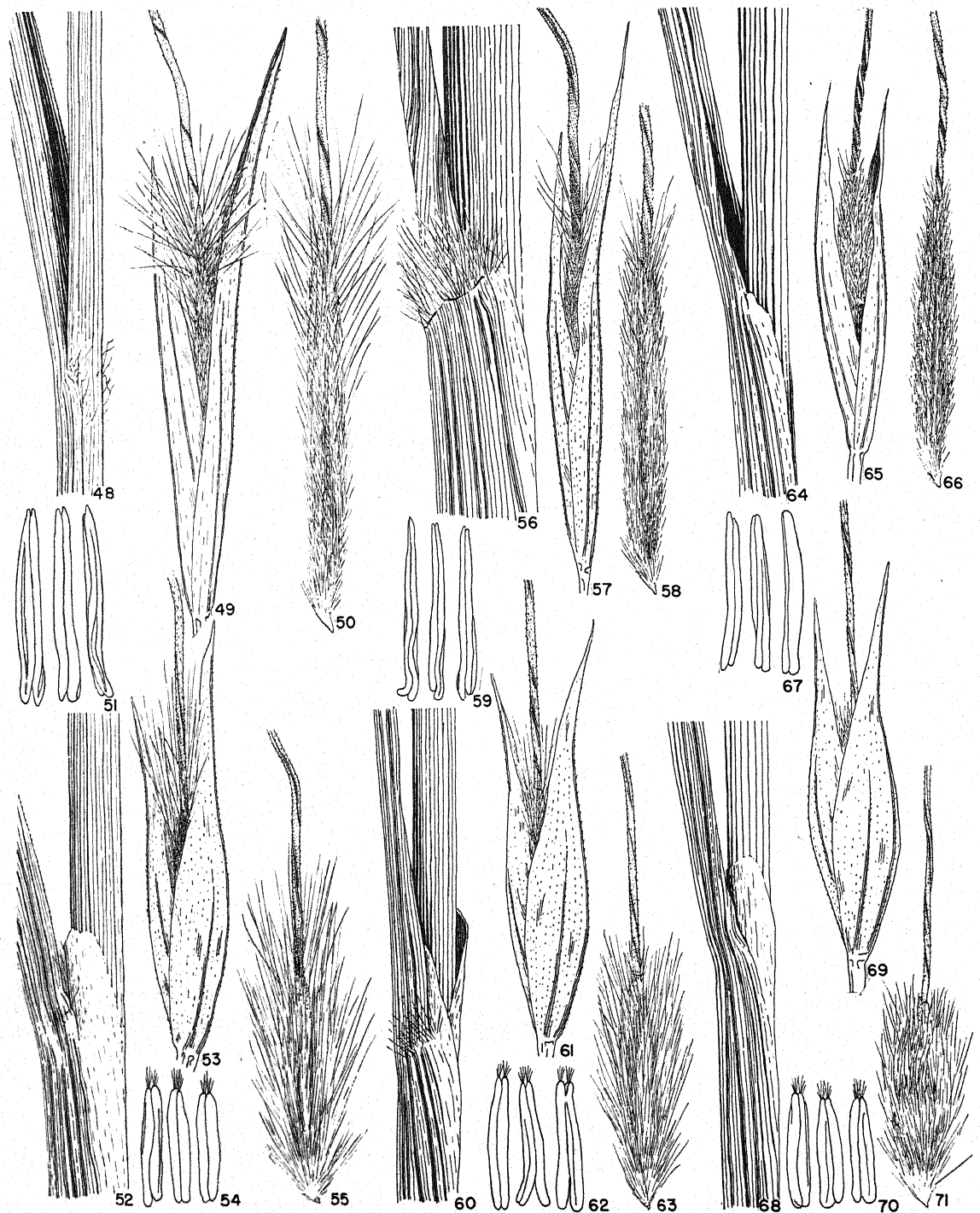


Fig. 48-71. Floral and vegetative parts.—Fig. 48-51. *Stipa Scribneri*.—Fig. 48. Throat of sheath.—Fig. 49. Spikelet.—Fig. 50. Lemma.—Fig. 51. Anthers.—Fig. 52-55. (*O. hymenoides* \times *S. Scribneri*) type.—Fig. 52. Ligule and throat of sheath.—Fig. 53. Spikelet.—Fig. 54. Anthers.—Fig. 55. Lemma.—Fig. 56-59. *Stipa robusta*.—Fig. 56. Throat of sheath.—Fig. 57. Spikelet.—Fig. 58. Lemma.—Fig. 59. Anthers.—Fig. 60-63. (*O. hymenoides* \times *S. robusta*) type.—Fig. 60. Ligule and throat of sheath.—Fig. 61. Spikelet.—Fig. 62. Anthers.—Fig. 63. Lemma.—Fig. 64-67. *Stipa columbiana*.—Fig. 64. Throat of sheath.—Fig. 65. Spikelet.—Fig. 66. Lemma.—Fig. 67. Anthers.—Fig. 68-71. (*O. hymenoides* \times *S. columbiana*) type.—Fig. 68. Ligule and throat of sheath.—Fig. 69. Spikelet.—Fig. 70. Anthers.—Fig. 71. Lemma. All $\times 8$.

A specimen of this hybrid and a specimen of *Stipa Thurberiana* were collected by Sandberg and Leiberg at Wilson Creek, Washington. The labels give the same date and elevation for both specimens. Another specimen of this hybrid was collected by Griffiths and Hunter (No. 341) in eastern Oregon. The locality is cited as "Bedes Buttes to Shirk's Ranch." Hitchcock (1925) cites a specimen of *S. Thurberiana* by the same collectors (No. 354) from "Bedes Buttes." Both of these localities are well within the range of *S. Thurberiana* (fig. 4).

The following specimens provided the data for *Stipa Thurberiana* and (*O. hymenoides* × *S. Thurberiana*) included in tables 1, 2 and 4:

Stipa Thurberiana. Washington: Wilson Creek, 1893, Sandberg and Leiberg (UM). Idaho, Blaine County, McBride and Payson 2962 (MBG).

(*O. hymenoides* × *S. Thurberiana*). Washington: Wilson Creek, Sandberg and Leiberg 231 (UM) Type. Oregon: "Bedes Butte" (Probably, Beaties Butte), Griffiths and Hunter 341 (US).

5. (***Oryzopsis hymenoides* × *Stipa californica***).
hybr. nov., aristis sub-plumosis, vaginis glabris, ligulis minoribus quam 2 mm. longis, villis partis superae lemmatis pluribus quam 2 mm. longis.

The hybrid (fig. 44-47) (tables 2 and 4) between *Stipa californica* Merr. and Davy. (fig. 40-43) (tables 2 and 4) and *O. hymenoides* again is very similar to × *Stiporyzopsis Bloomeri*. The awn is sub-plumose; the sheath is glabrous, or sparsely pubescent on the back; the ligule is slightly longer, and the plant is sterile as to pollen and seed. It differs distinctly from × *Stiporyzopsis Bloomeri* in the length of the hairs on the lemma. These are about 2.3 mm. long on the upper part of the lemma suggesting the condition in *S. californica* where the hairs on the upper part of the lemma are 1.8 mm. long as compared with 0.6 in *S. occidentalis*. Also the edge of the sheath in (*O. hymenoides* × *S. californica*) is ciliate as in the *Stipa* parent.

This hybrid is represented by a single specimen collected in Mono County, California, where the ranges of *S. californica* (fig. 5) and *O. hymenoides* overlap.

The following specimens provided the data for *S. californica* and (*O. hymenoides* × *S. californica*) in tables 1, 2 and 4:

Stipa californica. California: Humboldt County, Tracy 10446 (UC); Yosemite Park, Hall and Babcock 3336 (UC).

(*O. hymenoides* × *S. californica*). California: Mono County, Keck 3892 (UC) Type.

6. (***Oryzopsis hymenoides* × *Stipa Scribneri***).
hybr. nov., aristis nudis, vaginis leviter villosis ad collem, villis lemmatis pluribus quam 2.5 mm. longis.

In size of floral parts and most of the other features of morphology the hybrid (fig. 52-55) (tables 2 and 5) between *Stipa Scribneri* Vasey (fig. 48-51) (tables 2 and 5) and *O. hymenoides* is comparable with × *Stiporyzopsis caduca* and with the

group of hybrids with sub-plumose awns. Like all of these it is pollen sterile and shows no seed set. Its glabrous awn, however, allies it with × *Stiporyzopsis caduca* and clearly separates it from the others. Like × *Stiporyzopsis caduca* it also has a sparsely villous, sometimes glabrous, throat of the sheath. It may, however, be distinguished by the long hairs on the upper part of the lemma which are about 3.0 mm. long. In this feature it shows affinity to *S. Scribneri* which has hairs 2.2 mm. long near the summit of the lemma. The hybrid has five nerves in the lemma as does also *S. Scribneri*, while × *Stiporyzopsis caduca* has seven. It differs also from the latter in having equal, non-abortive anthers. The first and second glumes of the hybrid are quite different in length suggesting the distinctly different length of glumes in *S. Scribneri*. In the length of hairs on the lemma, the difference in glume length and the condition of the throat of the sheath the hybrid is intermediate between *S. Scribneri* and *O. hymenoides*.

The only specimen of this hybrid studied was collected by Shear (No. 21) at Trinidad, Colorado, within the region of overlap of the ranges (fig. 6) of the two parents. Hitchcock (1925) cites a specimen of *S. Scribneri* collected by Shear (No. 16) at Trinidad, Colorado.

The following specimens provided the data for *S. Scribneri* and (*O. hymenoides* × *S. Scribneri*) in tables 1, 2 and 5:

Stipa Scribneri. Colorado: Empire, Patterson 273 (UM). Texas: Culberson County, Moore and Steyermark 3638 (UM).

(*O. hymenoides* × *S. Scribneri*). Colorado: Trinidad, Shear 21 (US) Type.

7. (***Oryzopsis hymenoides* × *Stipa robusta***).
hybr. nov., aristis nudis, vaginis interdum villosis ad collem, villis lemmatis ca. 2 mm. longis.

The hybrid (fig. 60-63) (tables 2 and 5) between *Stipa robusta* Scribn. (fig. 56-59) (tables 2 and 5) and *O. hymenoides* is distinguishable from × *Stiporyzopsis caduca* on minor characters as may be expected since the *Stipa* parents are hardly distinguishable where their ranges come in contact. In both the awn is glabrous. However, some of the sheaths in (*O. hymenoides* × *S. robusta*) are quite villous at the throat or show a hispidulous line across the collar as does *Stipa robusta*. The floral parts in general are larger than in × *Stiporyzopsis caduca* suggesting the difference in size between those of the *Stipa* parents. The hairs of the lemma in (*O. hymenoides* × *S. robusta*) are almost twice as long as in × *Stiporyzopsis caduca*. In *S. robusta* they are also at least twice as long as in *S. viridula*. The anthers of (*O. hymenoides* × *S. robusta*) are much longer and are of equal size while in × *Stiporyzopsis caduca*, as previously pointed out, the anthers are short, probably somewhat abortive, and dimorphous. Similar conditions exist in their respective *Stipa* parents with respect to anther length. *Stipa robusta* commonly has 5 nerves in the lemma

with occasionally one or two additional ones developed. The same is true for its hybrid.

This hybrid is represented in this study by two specimens collected where the ranges (fig. 7) of *S. robusta* and *O. hymenoides* overlap. One of these (Hitchcock 22988) was collected at Glorieta, New Mexico. Hitchcock (1925) cites a collection of *S. robusta* (Griffiths 5040) from "Glorietta Mountains," New Mexico.

The following specimens provide the data for *S. robusta* and (*O. hymenoides* \times *S. robusta*) in tables 1, 2 and 5:

Stipa robusta. New Mexico: White Mountains, Wooton 370 (UM) Colorado: Manitou, Clements 17 (UM).

(*O. hymenoides* \times *S. robusta*). New Mexico: Glorieta, Hitchcock 22988 (US) Type. Arizona: Grand Canyon, Silveus 1927 (US).

8. (***Oryzopsis hymenoides* \times *Stipa columbiana***). hybr. nov., aristis nudis, vaginis glabris ad collem, villis lemmatis ca. 2 mm. longis.

This hybrid (fig. 68-71) (tables 2 and 5) in size of floral parts and in most other features of morphology is very similar to \times *Stiporyzopsis caduca*, and is somewhat smaller than (*O. hymenoides* \times *S. robusta*). It has a glabrous awn. It differs from both of these in having a distinctly glabrous throat of the sheath. This character especially separates it from (*O. hymenoides* \times *S. robusta*) in which some of the sheaths are villous at the throat or hispidulous on the collar. The well developed, equal anthers, the longer hairs on the lemma, and the five nerves of the lemma further distinguished it from \times *Stiporyzopsis caduca*. These characters are traceable to *Stipa columbiana* Macoun. (fig. 64-67) (tables 2 and 5).

The only specimen of (*O. hymenoides* \times *S. columbiana*) examined in this study was collected in Garfield County, Utah, beyond the range of *Stipa viridula* and at a higher altitude (7000 ft.). This locality is well within the range of *S. columbiana* (fig. 8), which also occurs at high altitudes in contrast to *S. viridula* and *S. robusta* which are both species of the dry plains.

The following specimens provided the data for *S. columbiana* and (*O. hymenoides* \times *S. columbiana*) in tables 1, 2 and 5:

Stipa columbiana. Wyoming: Cascade Creek, Nelson 6677 (UM) Oregon: Wallowa Mountains, Cusick 3195 (UM).

(*O. hymenoides* \times *S. columbiana*). Utah: Garfield County, Stanton 638 (US) Type.

DISCUSSION.—The concept of *O. Bloomeri* as a species presented difficulties which increased with the collection of more specimens. The specimens are rare in occurrence, but instead of being endemic to any restricted area they have a wide geographic

distribution. They lack generic characters which effectively separate them from either *Oryzopsis* or *Stipa*. At the same time they possess sufficient variation to have permitted the description of two species on the basis of the first two specimens collected. With an appreciation of the fact that these specimens constitute a series of parallel sterile hybrids involving the widely distributed *Oryzopsis hymenoides* and a number of species of *Stipa*, the entire problem is readily understood.

SUMMARY

Evidence is presented to show that *Oryzopsis hymenoides* crosses naturally with several species of *Stipa* to produce sterile hybrids which have in the past been classified under the specific name of *Oryzopsis Bloomeri*.

The hybrids are similar in characters which are intermediate between *O. hymenoides* and these species of *Stipa*. They are distinct in characters which reflect a different *Stipa* parent in each case.

Each hybrid was collected where the range of *O. hymenoides* overlaps that of its *Stipa* parent.

The type specimen of *O. Bloomeri* (Boland.) Ricker is identified as a hybrid between *O. hymenoides* and *Stipa occidentalis*. The hybrid name and formula, \times *Stiporyzopsis Bloomeri* (Boland.) (*Oryzopsis hymenoides* \times *Stipa occidentalis*) are applied to it.

The type specimen of *Oryzopsis caduca* Beal, which was included in *O. Bloomeri* by Hitchcock, was identified in an earlier paper as a hybrid and given the name and formula \times *Stiporyzopsis caduca* (Beal) Johnson and Rogler (*Oryzopsis hymenoides* \times *Stipa viridula*).

Six additional hybrids are identified and described here under the following formulas:

- (*O. hymenoides* \times *S. Elmeri*)
- (*O. hymenoides* \times *S. Thurberiana*)
- (*O. hymenoides* \times *S. californica*)
- (*O. hymenoides* \times *S. Scribneri*)
- (*O. hymenoides* \times *S. robusta*)
- (*O. hymenoides* \times *S. columbiana*).

RUBBER PLANT INVESTIGATIONS,
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL
ENGINEERING,
SALINAS, CALIFORNIA

LITERATURE CITED

- HITCHCOCK, A. S. 1925. The North American species of *Stipa*. Contrib. U. S. National Herb. 24: 215-289.
- . 1935. Manual of the grasses of the United States. U. S. Dept. Agric. Misc. Publ. No. 200.
- JOHNSON, B. L., AND G. A. ROGLER. 1943. A cytotoxic study of an intergeneric hybrid between *Oryzopsis hymenoides* and *Stipa viridula*. Amer. Jour. Bot. 30: 49-56.

THE KNOWN GEOGRAPHIC DISTRIBUTION OF THE MEMBERS OF THE VERBENACEAE AND AVICENNIACEAE: SUPPLEMENT 4¹

Harold N. Moldenke

OVER 3800 additional herbarium specimens of *Verbenaceae* and *Avicenniaceae* have been examined by the writer since the preparation of the last supplement to his listing of the known geographic distribution of the 2980 valid members of these two plant families based on an examination of 75,000 specimens.² This additional material has come from the herbaria of the New York Botanical Garden, Trinidad and Tobago Botanical Garden, United States National Museum, Instituto Ciencias Naturales at Bogotá, Butler University, Duke University, West Virginia University, Arnold Arboretum, Carnegie Museum, Kansas State College, Colorado State College, Pomona College, Georgia Agricultural Experiment Station, and numerous individual collectors, including Dr. H. Hapeman at Minden, Nebraska. It has yielded the following 53 new country or island records, 34 state or province records, and 141 county records. One new species and one new combination are proposed.

NEW YORK: *Phyla lanceolata* (Michx.) Greene—Onondaga County. *Verbena hastata* L.—Erie County. *Verbena urticifolia* L.—Suffolk County.

PENNSYLVANIA: *Phyla lanceolata* (Michx.) Greene—Butler County. *Verbena canadensis* (L.) Britton—Washington County. \times *Verbena Engelmannii* Moldenke—Crawford and Delaware Counties. *Verbena hastata* L.—Allegheny, Beaver, Blair, Clarion, Crawford, Elk, Forest, Indiana, Jefferson, McKean, Mercer, Potter, Somerset, Tioga, Union, and Venango Counties. *Verbena hastata* f. *rosea* Cheney—Huntingdon County. *Verbena simplex* Lehm.—Bedford, Erie, and Fulton Counties. *Verbena stricta* Vent.—Allegheny, Erie, and Westmoreland Counties. *Verbena urticifolia* L.—Beaver, Bedford, Bradford, Butler, Cambria, Centre, Clarion, Clearfield, Lawrence, Pike, and Westmoreland Counties. *Verbena urticifolia* var. *leiocarpa* Perry & Fernald—Clearfield, Greene, Lawrence, Northampton, Washington, Wayne, and Westmoreland Counties.

MARYLAND: *Phyla lanceolata* (Michx.) Greene—Washington County.

WEST VIRGINIA: *Verbena hastata* L.—Cabell, Kanawha, and Raleigh Counties. *Verbena simplex* Lehm.—Kanawha County. *Verbena urticifolia* L.—Marion County. *Verbena urticifolia* var. *leiocarpa* Perry & Fernald—Barbour and Randolph Counties.

¹ Received for publication July 14, 1945.

² Moldenke, H. N., The known geographic distribution of the members of the *Verbenaceae* and *Avicenniaceae*. 104 pp., New York City. 1942.

———. *Ibid.* Supplement 1. 4 pp., New York Botanical Garden. 1943.

———. *Ibid.* Supplement 2. Botanical Gazette 106: 158–164. 1944.

———. *Ibid.* Supplement 3. Castanea 10: 35–46. 1945.

NORTH CAROLINA: *Callicarpa americana* L.—Dare County.

GEORGIA: *Callicarpa americana* L.—Camden, Catoosa, Clayton, Colquitt, and Liberty Counties. *Phyla nodiflora* (L.) Greene—Tift County. *Stylo-don carneus* (Medic.) Moldenke—Tift County. *Verbena Halei* Small—Turner County. *Verbena rigida* Spreng.—Pike County. *Verbena simplex* Lehm.—Clayton County. *Verbena tenuisecta* Briq.—Colquitt County.

FLORIDA: *Lantana Camara* L.—Charlotte, Dade, and Polk Counties. *Lantana Camara* var. *aculeata* (L.) Moldenke—Hillsborough County. *Lantana involucrata* L.—De Soto County. *Lantana montevidensis* (Spreng.) Briq.—Polk County. *Sty-lodon carneus* (Medic.) Moldenke—Jackson County. *Verbena maritima* Small—De Soto County.

MISSISSIPPI: *Phyla lanceolata* (Michx.) Greene—Harrison County. *Verbena bonariensis* L.—Harrison County. *Verbena canadensis* (L.) Brit-ton—Pike County.

OHIO: *Phyla lanceolata* (Michx.) Greene—Ot-tawa and Wayne Counties. *Verbena hastata* L.—Ottawa County. *Verbena urticifolia* L.—Belmont, Richland, Tuscarawas, and Warren Counties.

ILLINOIS: *Phyla lanceolata* (Michx.) Greene—Henderson and Tazewell Counties. *Verbena bracteata* Lag. & Rodr.—Henderson County. \times *Ver-bena Engelmannii* Moldenke—Tazewell County. *Verbena hastata* L.—Tazewell County. *Verbena simplex* Lehm.—McLean County. *Verbena urticifo-lia* L.—Tazewell County.

IOWA: *Phyla lanceolata* (Michx.) Greene—Jackson County. *Verbena bracteata* Lag. & Rodr.—Dickinson and Dubuque Counties.

KENTUCKY: *Phyla lanceolata* (Michx.) Greene—Anderson County. *Verbena bracteata* Lag. & Rodr.—Anderson County. *Verbena simplex* Lehm.—An-derson County. *Verbena urticifolia* L.—Harlan County.

TENNESSEE: *Callicarpa americana* L.—Grun-dy County. *Phyla lanceolata* (Michx.) Greene—Obion County. *Verbena bracteata* Lag. & Rodr.—Obion County. *Verbena simplex* Lehm.—Claiborn County. *Verbena stricta* Vent.—Obion County. *Ver-bena urticifolia* L.—Obion County. *Verbena urti-ci-folia* var. *leiocarpa* Perry & Fernald—Obion County.

NORTH DAKOTA: *Verbena bracteata* Lag. & Rodr.—Cass County. *Verbena hastata* L.—Pem-bina County. *Verbena stricta* Vent.—Cass County. *Verbena ulticifolia* var. *leiocarpa* Perry & Fernald—*Verbena urticifolia* var. *leiocarpa* Perry & Fernald—Ransom County.

MISSOURI: *Callicarpa americana* L.—Chariton County. \times *Verbena Engelmannii* Moldenke—Linn County.

MISSOURI: *Callicarpa americana* L.—Chariton

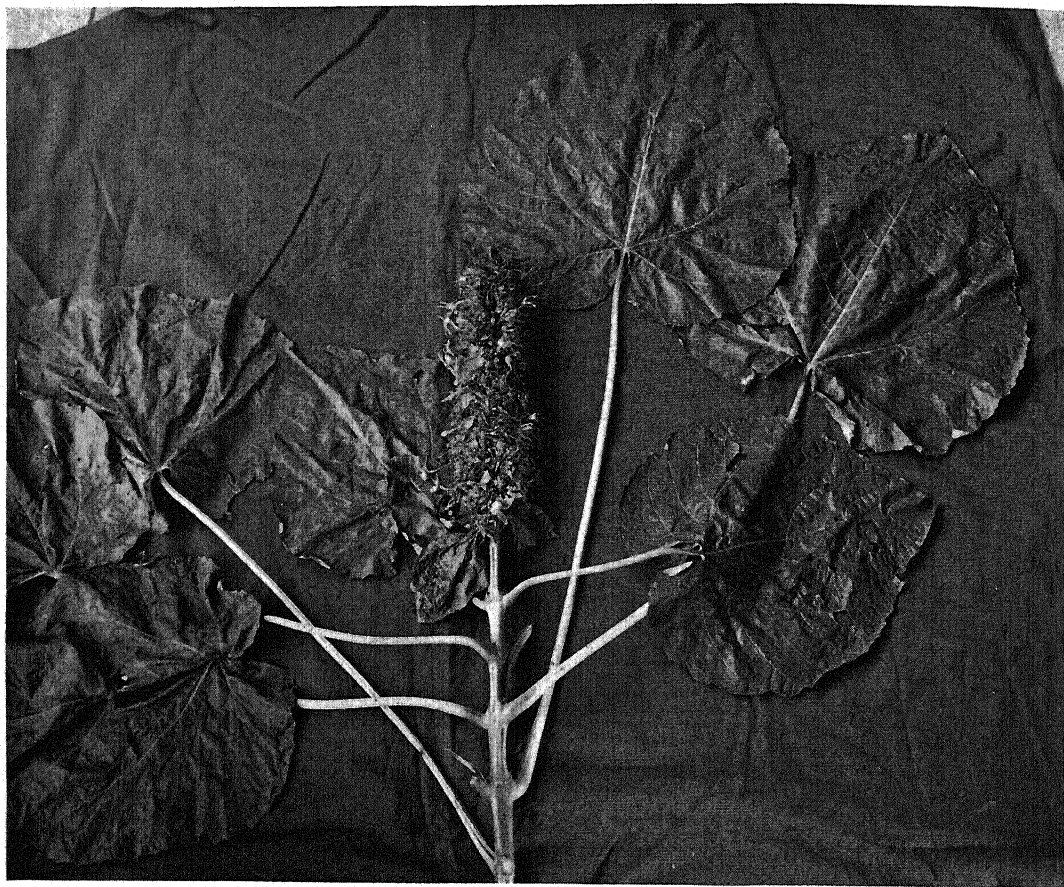


Fig. 1. *Clerodendrum spectabile*. Flowering branch before being dried; about $\frac{1}{8}$ natural size. Photo by Beckwith; courtesy of David Fairchild.

County.³ *Verbena canadensis* (L.) Britton—Bates County. \times *Verbena Perriana* Moldenke—the entry on page 9 of the original list should read: Jackson, Jasper, and Saint Louis Counties. *Verbena stricta* Vent.—Chariton County.

ARKANSAS: *Callicarpa americana* L.—Ashley County. *Phyla lanceolata* (Michx.) Greene—Randolph County. *Verbena bonariensis* L.—Logan County. *Verbena canadensis* (L.) Britton—Logan County.

WYOMING: *Verbena bracteata* Lag. & Rodr.—Hot Springs County.

UTAH: *Phyla cuneifolia* (Torr.) Greene—Sevier County. *Verbena bracteata* Lag. & Rodr.—Tooele and Utah Counties.

COLORADO: *Verbena hastata* L.—Arapahoe County. *Verbena MacDougalii* Heller—La Plata County.

³ This record finally substantiates the hitherto unverified statements of N. L. Britton, Robinson & Fernald, Baker, and F. S. Mathews, to the effect that this species grows wild in Missouri. This statement had been challenged by the present writer in his monograph of the genus (Fedde, Repert. Spec. No. 39:307, 1936) because of the absence of substantiating herbarium vouchers, and the following statement was made: "Collectors who have traversed all of Missouri inform the writer that it does not occur in that state."

NEBRASKA: *Verbena hastata* L.—Kearney County. *Verbena urticifolia* L.—Adams County.

TEXAS: *Tetradlea Coulteri* A. Gray—Hidalgo County. *Verbena canescens* var. *Roemeriana* (Scheele) Perry—Brooks County. *Verbena Halei* f. *albiflora* L. I. Davis—Brooks and Hidalgo Counties. *Verbena neomexicana* var. *xylopoda* Perry—Webb County.

ARIZONA: *Verbena ambrosifolia* Rydb.—Union County. *Verbena bipinnatifida* Nutt.—Navajo County. *Verbena ciliata* Benth.—Gila and Pima Counties.

WASHINGTON: *Verbena bracteata* Lag. & Rodr.—Franklin County.

CALIFORNIA: *Verbena ciliata* Benth.—Los Angeles County. *Verbena lasiostachys* var. *septentrionalis* Moldenke—Contra Costa County.

MEXICO: *Aegiphila Deppeana* Steud.—Puebla. *Lippia Pringlei* Briq.—Durango. *Phyla cuneifolia* (Torr.) Greene—San Luis Potosí. *Vitex mollis* H.B.K.—Veracruz.

GUATEMALA: *Cornutia grandifolia* var. *intermedia* Moldenke—Amatitlan and Sacatepéquez.

NICARAGUA: *Cornutia grandifolia* (Schlecht. & Cham.) Schau.—Chontales.



Fig. 2. *Clerodendrum spectabile*. Photograph of the type specimen in herbarium, Arnold Arboretum; about $\frac{3}{4}$ natural size. Photo courtesy of New York Botanical Garden.

CUBA: *Lantana Camara* var. *mista* (L.) L. H. Bailey—Havana.

MONTSERRAT: *Stachytarpheta jamaicensis* (L.) Vahl.

COLOMBIA: *Clerodendrum fragrans* var. *pleniflorum* Schau.—Chocó. *Petrea aspera* Turcz.—Magdalena. *Phylla nodiflora* (L.) Greene—Magdalena. *Vitex compressa* Turcz.—Atlántico.

VENEZUELA: *Lantana Moritziana* Otto & Dietr.—Bolívar and Lará.

ECUADOR: *Vitex flavens* H.B.K.—El Oro and Los Ríos.

PERU: *Aegiphila multiflora* Ruiz & Pav.—Madre de Dios.

BRAZIL: *Lippia hermannioides* Cham.—Goyaz.

CANARY ISLANDS: *Verbena officinalis* L.—Gran Canaria.

SPAIN: *Lantana Camara* L.

SOUTHERN NIGERIA: *Stachytarpheta cayennensis* (L. C. Rich.) Vahl.

CAMEROONS: On page 47 of the original list change "*Clerodendrum Preussii* Gürke" to read "*Clerodendrum Silvaeaeanum* Henriq."

FERNANDO PO: *Clerodendrum Silvaeaeanum* Henriq. *Clerodendrum volubile* P. Beauv.

ST. THOMAS: *Avicennia africana* P. Beauv. *Clerodendrum speciosissimum* Van Geert. *Clerodendrum umbellatum* Poir. *Duranta repens* L. *Lantana Camara* L.

PRINCIPE: *Clerodendrum Thomasii* Moldenke. *Clerodendrum volubile* P. Beauv.

ANNOBON ISLAND: On page 48 of the original list change "*Clerodendrum Preussii* Gürke" to read "*Clerodendrum Silvaeaeanum* Henriq."

UNION OF SOUTH AFRICA: *Lippia scabra* Hochst.—Transvaal.

AFGHANISTAN: *Vitex trifolia* L.

INDIA: *Holmskioldia sanguinea* Retz.—Bengal.

PHILIPPINE ISLANDS: *Avicennia marina* var. *Rumphiana* (H. Hallier) Bakh.—Jolo. *Callicarpa longifolia* Lam.—Cebu. *Clerodendrum minahassae* Teijsm. & Binn.—Busuanga. *Geunsia farinosa* Blume—Mindanao and Sulu. *Premna nauseosa* Blanco—Corregidor. *Tectona grandis* L. f.—Basilan. *Vitex quinata* (Lour.) F. N. Will.—Palawan. *Vitex trifolia* var. *bicolor* (Willd.) Moldenke—Mindanao.

SUMATRA: *Geunsia furfuracea* (Bakh.) Moldenke.

BRITISH NORTH BORNEO: *Geunsia farinosa* Blume.

BORNEO: *Geunsia furfuracea* (Bakh.) Moldenke.

CELEBES: *Geunsia furfuracea* (Bakh.) Moldenke.

LESSER SUNDA ISLANDS: *Geunsia furfuracea* (Bakh.) Moldenke—Banka.

MOLUCCA ISLANDS: *Clerodendrum spectabile* Moldenke—Manipa.⁴ *Geunsia farinosa* Blume—Amboina.

NEW GUINEA: *Geunsia Cumingiana* (Schau.) Rolfe—Papua. *Geunsia farinosa* Blume—Papua.

HAWAIIAN ISLANDS: *Lantana Camara* L.—Oahu. *Stachytarpheta cayennensis* (L. C. Rich.) Vahl—Oahu. *Stachytarpheta urticaefolia* (Salisb.) Sims—Hawaii and Oahu.

SOLOMON ISLANDS: *Geunsia furfuracea* (Bakh.) Moldenke—Guadalcanal and Malaita.

FIJI ISLANDS: *Stachytarpheta urticaefolia* (Salisb.) Sims—Vanua Levu.

AUSTRALIA: *Clerodendrum Holtzei* F. Muell.—Northern Territory. *Premna acuminata* R. Br.—Northern Territory.

CULTIVATED: *Callicarpa formosana* Rolfe—

Philippine Islands. *Clerodendrum indicum* (L.) Kuntze—Georgia. *Clerodendrum Thomsonae* Balf.

f.—Honduras and Pennsylvania. *Congea tomentosa* Roxb.—Honduras. *Duranta repens* var. *alba* (Masters) L. H. Bailey—Honduras. *Geunsia Cumingiana* (Schau.) Rolfe—Florida. *Gmelina philippensis* Cham.—Thailand. *Lantana Camara* var. *aculeata* (L.) Moldenke—Nebraska. *Lantana montevidensis* (Spreng.) Briq.—Honduras. *Premna odorata* Blanco—Philippine Islands. *Premna subglabra* Merr.—Florida. *Verbena tenuisecta* Briq.—Italy. *Vitex Agnus-castus* var. *caerulea* Rehd.—North

⁴ Endemic; as far as is now known.

Carolina. *Vitex Negundo* var. *incisa* (Lam.) C. B. Clarke—Arizona.

Geunsia furfuracea (Bakh.) Moldenke, comb. nov.

Callicarpa pentandra var. *paloensis* f. *furfuracea* Bakh. in Lam. & Bakh., Bull. Jard. Bot. Buitenz., ser. 3, 30: 15. 1921.

CLERODENDRUM spectabile Moldenke, sp. nov. figure 1 and 2.

Frutex; ramis percrassis medullosis tetragonis pulverulentis vel glabris; foliis magnis; petiolis 10–45 cm. longis glabris; laminis foliorum membranaceis cordato-ovatis 22–36 cm. longis latisque acuminatis remote sinuato-denticulatis, ad basin profunde cordatis, supra parce pilosulis vel glabrescentibus, subtus glabrescentibus punctatis; inflorescentiis racemiformibus densissimis terminalibus usque ad 60 cm. longis et 13 cm. latis.

Shrub, about 2.5 m. tall; branches very stout and medullose, tetragonal, decussately compressed, pulverulent or glabrate; leaves decussate-opposite, very large; petioles stout, 10–45 cm. long, glabrate, collapsing at base in drying; blades thin-membranous, dark-green above, lighter beneath, cordate-ovate, 22–36 cm. long and wide, acuminate at apex, remotely sinuate-denticulate along the margins, deeply cordate at base, very sparsely pilosulous or glabrescent above, glabrescent and punctate beneath; midrib flat above, prominent beneath; secondaries 4, issuing from the apex of the petiole in palmate fashion, 8–10 smaller ones issuing from the midrib at more or less irregular intervals to the apex; tertiaries slender, numerous, connecting the secondaries, more or less parallel; veinlet reticulation rather

obscure on both surfaces; inflorescence an extremely dense terminal raceme to 60 cm. long and 13 cm. wide, practically uniform in diameter throughout; peduncles apparently abbreviated, resembling the branches in all respects; bracts and bractlets extremely numerous, red-brown, the larger ones keel-shaped, elliptic or lanceolate, to 4 cm. long and 2.5 cm. wide, acuminate, glabrate, the smaller ones linear-elongate; flowers in individual clusters of about 20, interspersed with many undeveloped ones and with two or more long bracts per cluster; calyx red, glabrate, in fruit pure lacquer-red, its five lobes lanceolate, to 2 cm. long, broadest at base, long-acuminate at apex; corolla large, lighter red than the calyx, the tube slender, to 4 cm. long, the limb bilabiate, the lower lip 2-parted, the upper lip 3-lobed; corolla in bud "with the shape of a tennis racket, flat, with the stamens curved around in its upper part"; stamens 4, about 2 cm. long, curved, red; anthers introrse, dehiscing longitudinally; stigma bifid, green.

The type of this most remarkable and distinctive species was collected by Hugo Curran (*Fairchild Garden Expedition No. 317*) in sunny spots at sea level in the forest at the edge of a sago swamp on the northeast coast and "on top of the island," Maniapa, Molucca Islands, May 6, 1940, and is deposited in the herbarium of the Arnold Arboretum. Dr. David Fairchild says of it in his original notes: "The most amazing species of this genus that I have ever seen, having the showiest spikes of flowers of any kind I ever saw."

NEW YORK BOTANICAL GARDEN,
NEW YORK 58, NEW YORK

ABCDEFGHIJKLMNQRSTUWVWXYZ
1234567890

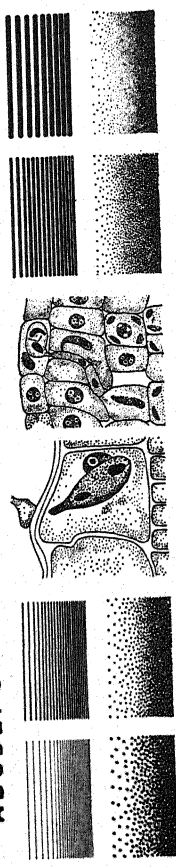
ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ



ABCDEFGHIJKLMNQRSTUWVWXYZ
1234567890

ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ

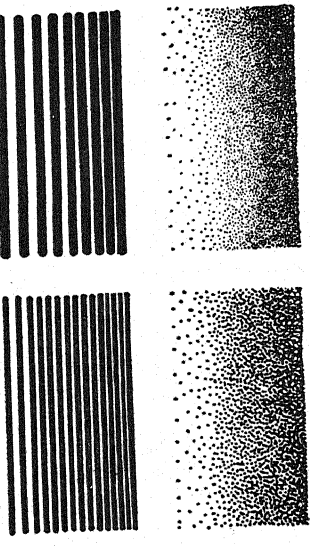
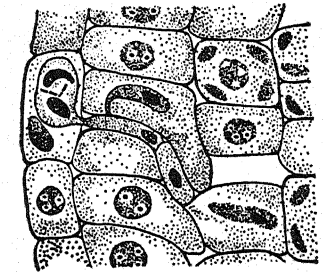
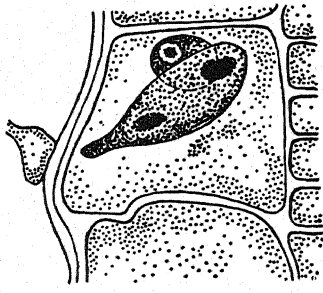
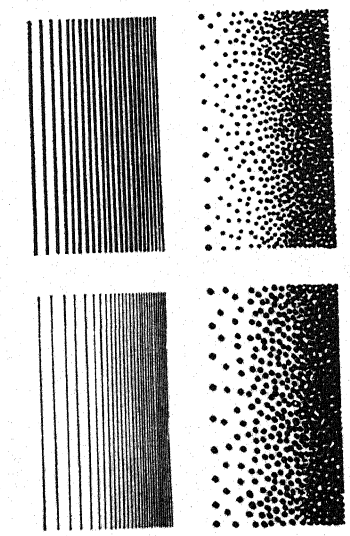
ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ



ABCDEFGHIJKLMNQRSTUWVWXYZ
1234567890

ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ

ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ
ABCDEFGHIJKLMNQRSTUWVWXYZ



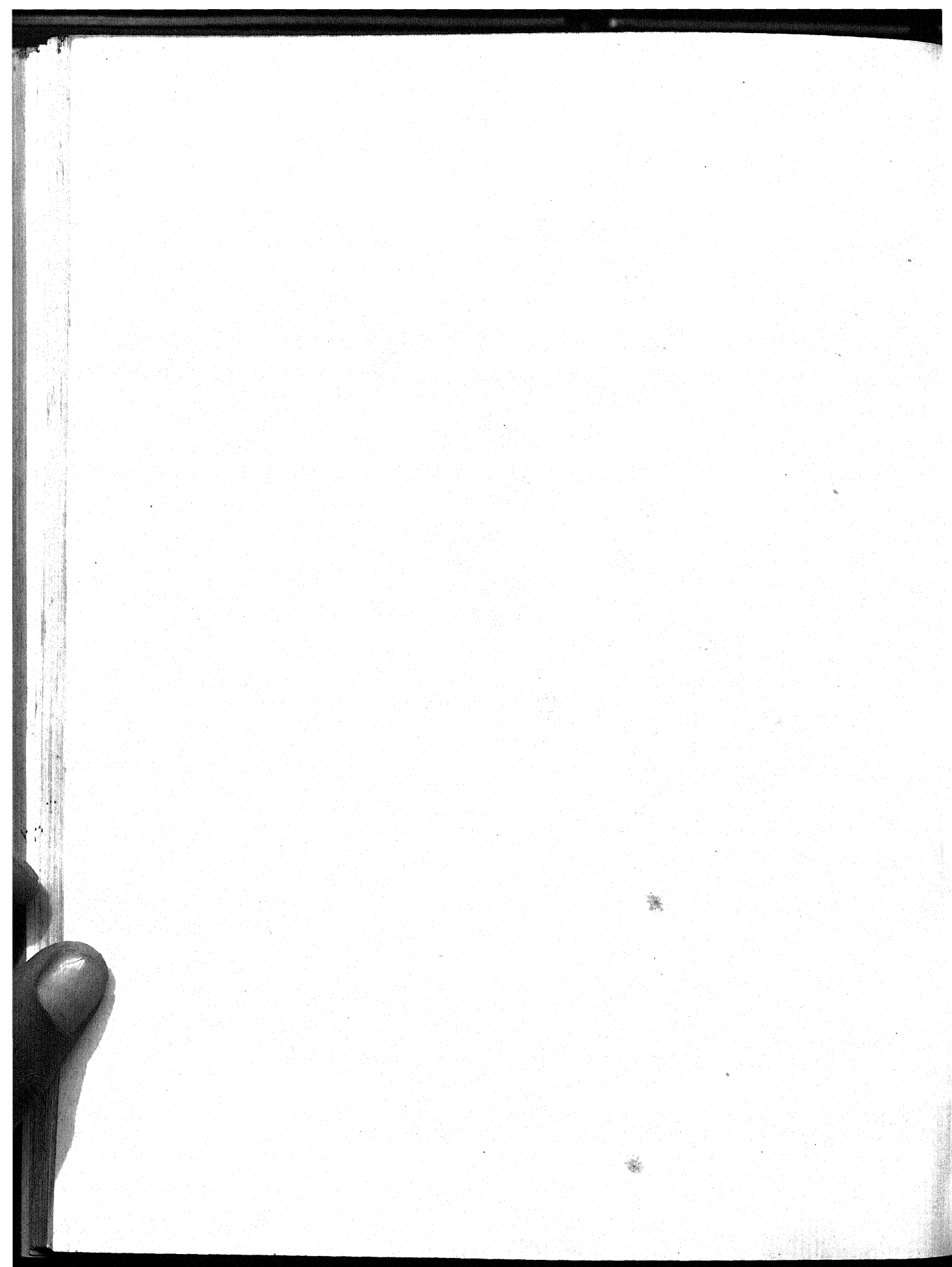
DRAWING GUIDE FOR ZINC ETCHINGS

Letters, lines, and dots in relation to reduction for the printed page. Top—Reduction to 1/4. Middle—Reduction to 1/2. Bottom—Original size.

Reprinted by permission of the authors from: RIKER, A. J., and REGINA S. RIKER. 1936. *Introduction to research on plant diseases*. 117 p. Published by the Authors, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Thin black lines may be shortened, but otherwise hold up fairly well in reduction, but small black dots and small white spaces between black lines or dots may be lost. If placed too close together, dots and lines produce black blotches when the drawing is reduced. The shading should be rather open. The degree of reduction needs to be known before the drawing is inked in.

Delicate shading may be obtained if the size and spacing of the dots are adjusted to the degree to which the drawing is to be reduced.



ACCURACY OF THE LOCAL-LESION METHOD FOR MEASURING VIRUS ACTIVITY. IV. SOUTHERN BEAN MOSAIC VIRUS¹

W. C. Price

DETAILED PROCEDURES for estimating the relative potency of drugs from the response induced in test animals by injection of graded doses were worked out by Bliss and Marks (1939a and 1939b). These procedures make it possible to calculate not only the most probable relative activity of the drug in terms of a standard, but also the limits within which the true activity will lie for any given level of significance. In recent work (Spencer and Price, 1943; Price and Spencer, 1943a, 1943b), the procedures of Bliss and Marks were applied in determining the accuracy of measuring the relative activities of certain plant viruses. From the results it was found that the relative activities of tobacco mosaic, tobacco necrosis, and tobacco ringspot viruses could be estimated with errors not exceeding 10, 11, and 14 per cent, respectively, under the prevailing experimental conditions and that the standard error of estimate was a reliable measure of the experimental error. The results with alfalfa mosaic virus were less satisfactory; the standard error of estimate could not be counted upon to give a reliable estimate of the true error. The present paper describes the results obtained when essentially the same procedures were applied in measuring the relative activity of southern bean mosaic virus (*Marmor laesiofaciens* Zaumeyer and Harter).

THE VIRUS.—Southern bean mosaic virus was first described by Zaumeyer and Harter (1942, 1943) who found all tested varieties of bean (*Phaseolus vulgaris* L.) to be susceptible. In some varieties, reddish necrotic lesions about 1 mm. or less in diameter were produced on inoculated leaves, and the virus was localized in these lesions. In other varieties no symptoms appeared on inoculated leaves, but the virus became systemic and caused a mottling disease in the young leaves. More recently, a strain of the Blue Lake variety was found to develop a systemic necrosis when infected with the virus (Zaumeyer and Harter, 1944). Mottling type varieties serve as a means of propagating the virus and of obtaining it in relatively large quantities. Local lesion type varieties offer a means of determining the relative activity of virus samples used in experimental work. In the present work, the virus was propagated in the Bountiful variety of garden bean and its activity was determined by inoculation of the Early Golden Cluster variety.² The virus samples used in the rela-

tive activity experiments to be reported were all from one stock solution, originally from Bountiful bean plants, but obtained in highly purified form by a chemical treatment to be described in a later publication.

THE DILUTION CURVE.—It was pointed out by Youden, Beale, and Guthrie (1935), and independently by Bald (1937a), that the relationship between virus concentration and numbers of lesions produced on susceptible host plants by a number of different viruses could be expressed by an equation of the form

$$\frac{y}{N} = 1 - e^{-vnx}.$$

This equation was derived from the Poisson series on the assumption that a single virus particle is sufficient to cause infection, y being defined as the number of lesions obtained with dilution x of the virus, N the number of susceptible regions on the host plants, n the number of virus particles per cc., v a small volume whose exact size need not be known, and e the base for natural logarithms.

Bald (1937b) later showed that many virus dilution series failed to follow this simple equation precisely. For such cases, he (1937c) derived a second equation which was based on the assumption that in concentrated solutions the virus particles were aggregated but became dissociated on dilution. If the number of aggregates be taken as b , the simple equation assumes the form

$$\frac{y}{N} = 1 - e^{-vbx}$$

and the exponential may be solved in terms of vnx to give

$$vbx = \frac{\sqrt{1 + 4Kvnx} - 1}{2K},$$

in which K , the equilibrium constant, is in effect a measure of the distortion of the dilution series.

If, in the case of a particular virus, the simple equation always held, there would be a region in which the relationship between lesions and virus concentration would be expressed approximately by a straight line with unit slope. In this region, the number of lesions produced by a virus sample could be considered as an exact measure of its activity. Moreover, this region would extend into that portion of the dilution curve where the experimental error is small. On the other hand, if the more complex equation held for a particular virus, the straight line of unit slope relationship would hold only in the more dilute range of the curve where the experi-

¹ Received for publication June 13, 1945.

² Seed of this variety was obtained from Peter Henderson and Co., 35 Cortlandt Street, New York, N. Y. It seems likely that there may be more than one strain of the variety since Zaumeyer and Harter (1943) found Golden Cluster wax bean plants to respond to infection with southern bean mosaic virus by the production of systemic mottling symptoms rather than with local lesions as was found here.

mental error is relatively large. In the region of small experimental error the number of lesions could not be taken as an exact measure of virus activity.



Fig. 1. Photograph illustrating method of inoculating bean leaves with southern bean mosaic virus. (Photograph by J. A. Carlile.)

For precise measurements, therefore, it would be necessary to use a method, such as that of Bliss and Marks, in which the slope of the curve is experimentally determined for each activity test.

To determine the shape of the southern bean mosaic virus dilution curve, series of dilutions were used to inoculate leaves of Early Golden Cluster bean plants. The method of inoculation is illustrated in figure 1. A cheesecloth pad saturated with the test dilution was firmly rubbed over each leaf as it rested on the palm of the hand. In four tests, the dilutions were prepared with 0.1 M potassium phosphate buffer at pH 7. In the first two tests, freshly extracted juice of diseased Bountiful bean plants was used. In a third test the virus was a chemically purified sample, and in the fourth test it was a sample prepared by alternating cycles of high and low speed centrifugation, as will be described elsewhere. For these last two tests the dilutions are expressed in terms of gms./cc. of virus.

It was possible to fit the experimental points of the second test to the curve of the simple dilution equation with N taken as 10,000 and vn as 53.1. The more complex equation was resorted to for the other three tests. The observed and calculated numbers of lesions for the different virus concentrations of each of the four tests are summarized in table 1 and the theoretical curves are presented in figure 2. It is obvious by inspection that the fit of the data to the curves is not so good as might be desired. Nevertheless, it is clearly apparent that the relationship between virus concentration and numbers of lesions is a variable. The relationship is probably influenced by the virus samples employed, the host plants, and the environmental conditions.

In general the experimental error for lesion counts is least when the number of lesions per leaf is, on the average, not less than about 10 nor greater than about 200. This corresponds roughly to the region of the dilution curve where $\log(vnx)$ is not less than -1.75 nor greater than 0.25 . As seen in figure 2, the relationship between lesions and virus concentration over this range is represented by a

TABLE 1. Numbers of lesions produced on Early Golden Cluster bean plants with various concentrations of southern bean mosaic virus.

-Log ^a concentration	Test 1 ◇		Test 2 ◆		-Log ^b concentration	Test 3 Δ		Test 4 ○	
	Lesions (observed)	Lesions (calculated)	Lesions (observed)	Lesions (calculated)		Lesions (observed)	Lesions (calculated)	Lesions (observed)	Lesions (calculated)
	(30 leaves)		(30 leaves)			(48 leaves)		(48 leaves)	
0.5	11,356	10,376	3.0	7,404	7,868	21,601	22,694
1.0	10,355	8,005	11,962	10,000	3.5	10,234	6,888	19,879
1.5	6,022	5,414	16,218	10,000	4.0	4,880	5,030	15,570	14,857
2.0	2,699	3,269	8,102	9,950	4.5	3,664	3,002	9,613	10,105
2.5	2,078	1,775	8,086	8,136	5.0	1,523	1,507	6,713	6,040
3.0	783	878	3,158	4,120	5.5	380	608	2,706	3,252
3.5	569	378	1,812	1,548	6.0	244	215	1,278	1,490
4.0	141	161	311	518	6.5	30	73	653	670
4.5	50	48	151	166	7.0	35	23	343	251
5.0	163	53	7.5	10	6.5	188	85
5.5	13	17

^a In tests 1 and 2, expressed juice of diseased plants was used. The virus concentration of the undiluted juice is taken as unity.

^b In tests 3 and 4, purified virus preparations were used. Virus concentration is expressed in grams per cc.

curved line, the degree of curvature depending upon whether the particular data under consideration follow the simple exponential equation or the more complicated one. For practical purposes, however, a linear relationship may be assumed provided the interval between the most concentrated and least concentrated virus samples is not greater than about two log units. The slope of the line will vary from test to test and must therefore be determined from the data.

THE METHOD OF ASSAY.—In previous papers (Spencer and Price, 1943; Price and Spencer, 1943b), the method of calculating the relative activity of a virus preparation from data such as will be presented here was given in detail. The procedure employed in the case of southern bean mosaic virus was similar to that referred to as Scheme I in the previous papers. However, Scheme I was not followed exactly and for this reason the procedure followed in the present work will be discussed in more detail than otherwise would be necessary.

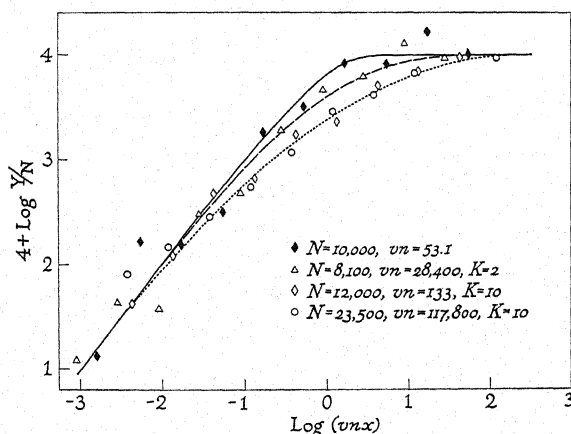


Fig. 2. Plot of the dilution curves for southern bean mosaic virus when Early Golden Cluster bean plants were used as test plants. (Photograph by J. A. Carlile.)

A purified preparation containing 5.45 mg./cc. of virus was used as the standard. It was diluted with 0.1 M potassium phosphate buffer at pH 7 to give preparations containing about 10^{-6} gm./cc. (S_1) and 10^{-5} gm./cc. (S_2). The preparations designated as unknowns were obtained from the standard by dilution with pH 7 phosphate buffer. Two dilutions of each unknown, with an interval of dilution of 1:10, were used and are referred to as U_1 and U_2 .

In each test, 18 pots, each containing three Early Golden Cluster bean plants, were used. Each plant bore two primary leaves, designated arbitrarily as leaf 1 and leaf 2. In the first three pots, S_1 was placed on the left half of leaf 1 and U_1 on the right half, while S_2 was put on the left half of leaf 2 and U_2 on the right half. In the second group of pots the leaf halves were reversed, S_1 and S_2 being placed on the right halves and U_1 and U_2 on the left halves. Pots 7 to 9 and 13 to 15 were replicates

of pots 1 to 3 while pots 10 to 12, and 16 to 18 were replicates of pots 4 to 6. Thus, each of the four virus preparations occurred as often on the left halves of the leaves as it did on the right halves. After five days the lesions were counted and tabulated.

The lesions produced by each of the four virus preparations on the three plants in each pot were summed. This gave a table with 18 counts for each virus suspension. In order to reduce the arithmetical labor, the data were still further aggregated in the following manner: the counts for the same preparations in pots 1 and 18 were added together, likewise those for pots 2 and 17, 3 and 16, 4 and 15, 5 and 14, 6 and 13, 7 and 12, 8 and 11, and 9 and 10, giving a table with nine entries for each of the four virus preparations and automatically adding together counts on the left and right halves of the leaves.

One complicating factor entered into the aggregation of the data in this manner. Occasionally a bean plant was found which, for some unknown reason, appeared to be completely refractory to infection; no lesions developed even after inoculation with the most concentrated virus samples. It was necessary to correct for such missing data. This was done by substituting for the missing value the mean of the remaining counts for the same treatment in the same block of pots. An error was thus introduced into the estimate of the relative activity and its standard deviation but, judged by the results, the error was not particularly significant.

Data from experiment 19, in which actual lesion counts were converted to logarithms, are summarized in table 2. These data will be used as an example to show how the relative concentration of the unknown and the standard error of estimate were calculated.

As given previously (Price and Spencer, 1943b)³, the equation for determining the relative activity of U in terms of S , or the log ratio of potencies, is

$$M = \frac{d \sum x^2}{b'N}$$

for an even number of dilutions. M is the log ratio of potencies, d is a factor for difference, b' is a factor for slope of the combined dilution curve, I is the interval in logarithms between dilutions, and N is the total number of dilutions. Since, in all the tests to be reported, $I = 1.0$, $\sum x^2 = 4$, and $N = 4$, the above equation reduces to

$$M = \frac{d}{b'}$$

The factor d is obtained by multiplying the treatment totals by the corresponding factorial coefficient for difference and summing the products. Similarly,

³ At the suggestion of the reviewer, the symbolism of the 1943 paper has here been modified to conform with that used in earlier papers by Bliss and Marks (1939a, 1939b) and others.

TABLE 2. Summary and analysis of the data of experiment 19 in which $U = 0.25 S$.

Lesion counts aggregated by pairs of pots and converted to logarithms					
Pots number	S_1	U_1	S_2	U_2	Pot total
1, 18	1.52	1.20	2.23	1.89	6.84
2, 17	1.63	1.00	2.09	2.02	6.74
3, 16	1.49	1.11	2.01	1.84	6.45
4, 15	1.63	1.34	2.23	1.94	7.13
5, 14	1.43	1.08	2.05	1.91	6.47
6, 13	1.40	1.11	1.91	1.90	6.32
7, 12	1.45	1.41	1.97	1.93	6.76
8, 11	1.40	1.34	2.03	1.73	6.50
9, 10	1.20	1.18	1.75	1.72	5.85
Treatment total	13.15	10.77	18.26	16.88	59.06
Factorial coefficients					
Difference (d)	-1	+1	-1	+1
Slope (b')	-1	-1	+1	+1
Opposed slope	+1	-1	-1	+1
Analysis of variance					
	D/F	SS	Variance		
Between leaves	17	3.8232			
Within leaves	18	.6400		
Total	35	4.4632			
Between pots	8	.2653			
Slope (B^2)	1	3.4969			
Error for B.....	8	.0610			.0076
Between leaves	17	3.8232			
Difference (D^2)	1	.3927			
Error for D.....	17	.2473			.0145
Within leaves	18	.6400			
Opposed slope	1	.0277			
Residual error	16	.2196			.0137
Error for D.....	17	.2473			

the factor b' is obtained by multiplying the same treatment totals by the factorial coefficients for slope and summing. In the example of table 2, $d = -3.76$, $b' = 11.22$, $M = -.3351$, and the anti-log of M , or the relative activity, is 0.462, where the true value is 0.5.

The true slope (b) of the combined dilution curve is equal to $2b'/N'\Sigma x^2$, where N' is the number of replicates and the other symbols have the same meaning as before. In the tests under consideration $N' = 9$, $I = 1.0$, $\Sigma x^2 = 4$, and therefore $b = 2b'/36 = b'/18$. For the data of table 2, $b = 11.22/18 = 0.62$.

PRESENTATION OF RESULTS.—The results of 26 experiments on accuracy of measuring southern bean mosaic virus are presented in table 3. There were four sets of experiments, in which U was 25 per cent, 35 per cent, 50 per cent, and 75 per cent of S . This is indicated in column A of the table. In preparing these unknowns, portions of the standard were measured with graduated pipettes and diluted with sufficient buffer to give the desired concentra-

tion. The error involved in the preparation is thought to have been small. Column B gives the estimate of the log ratio of potencies obtained from the experimental data and column F the calculated value of the estimated relative activity of U in terms of S . The true slope (b) of the combined dilution curve is given in column G.

It will be noted from column F that the activity of southern bean mosaic virus was estimated very accurately on the average. When U was 25 per cent of S the average inaccuracy of measurement was 15.6 per cent. The most accurate measurement was that of test 8 in which the error was 4 per cent and the least accurate was that of test 3 in which the error was 32 per cent. The four tests with $U = .35 S$ had an average error of 3.8 per cent and a maximum of 7 per cent. For the twelve tests with $U = .50 S$ the average error was 8.7 per cent with a minimum of less than 1 per cent (tests 14 and 22) and a maximum of 26 per cent (test 17). The error of measurement exceeded 10 per cent in only nine

TABLE 3. Summary of results of tests on accuracy of measuring southern bean mosaic virus.

Test number	True M (A)	Found M (B)	Difference (B-A) (C)	(S.E.) (D)	C/D = t (E)	Activity of U (F)	True slope b (G)
1	-.6021	-.5684	.0337	.0877	.38 ^b	.270	.40
2	-.6021	-.6387	-.0366	.0692	-.53	.230	.74
3	-.6021	-.7661	-.1640	.1016	-1.61	.171	.46
4	-.6021	-.6942	-.0921	.0800	-1.15	.202	.50
5	-.6021	-.5737	.0284	.0439	.65	.267	.60
6	-.6021	-.7129	-.1108	.0905	-1.22 ^c	.194	.57
7	-.6021	-.6853	-.0832	.0772	-1.08	.206	.74
8	-.6021	-.6240	-.0219	.0767	-.029	.238	.71
9	-.6021	-.5195	.0826	.0541	1.53	.302	.78
10	-.4559	-.4866	-.0307	.0384	-.80	.326	.68
11	-.4559	-.4368	.0181	.0485	.38	.366	.58
12	-.4559	-.4456	.0103	.0473	.22	.358	.75
13	-.4559	-.4571	-.0012	.0544	-.02	.349	.78
14	-.3010	-.2981	.0029	.0566	.05	.503	.49
15	-.3010	-.3680	-.0670	.0482	-1.39 ^b	.429	.77
16	-.3010	-.2531	.0479	.0372	1.29	.558	.58
17	-.3010	-.2043	.0967	.0503	1.92	.625	.62
18	-.3010	-.2970	.0040	.0576	.07	.502	.75
19	-.3010	-.3351	-.0341	.0663	-.51	.462	.62
20	-.3010	-.3289	-.0279	.0393	-.71	.469	.67
21	-.3010	-.3407	-.0397	.0656	-.61	.457	.65
22	-.3010	-.3035	-.0025	.0677	-.04	.497	.55
23	-.3010	-.2723	.0287	.0435	.66	.534	.83
24	-.3010	-.3297	-.0287	.0870	-.33	.468	.73
25	-.3010	-.3918	-.0908	.0389	2.33 ^a	.406	.68
26	-.1249	-.0979	.0270	.0394	.68	.798	.68

^a t value significant at .05 level^b Opposed slope significant at .05 level.^c Opposed slope significant at .01 level.

of the 26 tests, 15 per cent in only seven tests, and 20 per cent in only three tests.

There were sufficient numbers of tests at the 0.25 and 0.5 concentration levels to calculate the standard deviation of an estimate at these levels directly from the data of column F. At the 0.25 level, the standard deviation of a single observation is .044, and at the 0.5 level it is .057. Expressed on a per cent basis these standard deviations are 17.6 and 11.4 per cent, respectively. Thus it can be concluded that in experiments carried out by the method described the probability is 0.95 that the estimate should not differ from the true value of the unknown by more than 40 per cent when the true value is about 25 per cent of the standard, nor by more than 25 per cent when the true value is about 50 per cent of the standard. The agreement should be even better when dilutions are chosen such that the standard and unknown have approximately equal activities.

THE STANDARD ERROR OF ESTIMATE.—It was pointed out by Bliss and Marks (1939b) that the standard error of a single estimate could be calculated directly from the data. The question arises as to whether or not the standard error of estimate is a reliable measure of the accuracy of estimating the activity of southern bean mosaic virus in tests such as those described here. It was shown by Price and

Spencer (1943b) that the equation for calculating the standard error of the log ratio of potencies from experimental data such as are here being considered is

$$S_M = \frac{kI}{B^2} \sqrt{B^2 V_D + D^2 V_B}$$

where V_D and V_B represent the variance for the difference between the two virus samples and the variance for the slope of the combined dilution curve, respectively, $B^2 = b'^2/N'\Sigma(x)^2$, $D^2 = d^2/N'\Sigma(x')^2$, $k = \sqrt{\Sigma(x)^2/\Sigma(x')^2}$ for an even number of dilution, N' being the number of replicates, x the factorial coefficient for slope, and x' the factorial coefficient for difference. Since in the tests here considered $k = 1$ and $I = 1.0$,

$$S_M = \frac{1}{B^2} \sqrt{B^2 V_D + D^2 V_B}$$

V_D and V_B are obtained by analysis of variance as shown in the lower portion of table 2. It will be noticed that the estimate for V_D contains the variance for opposed slope. This was included to compensate for the fact that the estimate for M should be less accurate when the dilution curves for S and U are not parallel than when they are.

Table 2 gives $V_D = .0145$ and $V_B = .0076$. Sub-

stituting these values in the equation for S_M there is obtained

$$S_M = \frac{1}{3.4969} \left[3.4969(.0145) + .3927(.0076) \right]^{1/2} = .0663.$$

This can be multiplied by the appropriate t value for any desired level of significance to give the range within which the true value of M should be found for that level.

Column D of table 3 gives the standard error of estimate of each experiment, as determined by the above equation. The standard deviation of estimate may be calculated directly from the column of differences (column C) since there are nine such differences at the 0.25 level and 12 at the 0.5 level. The calculated values are .0851 and .0497, respectively, at the two levels. The means of the standard errors for the corresponding levels are .0756 and .0549, respectively. Thus it is seen that the mean of the standard errors calculated from the equation for S_M is a fair approximation of the standard deviation. However, the standard errors fluctuate widely about their means.

Several other tests may be applied in determining whether the standard error of estimate provides a reliable estimate of the true error. The quotients obtained when the differences of column C are divided by the corresponding standard errors are given in column E. These quotients are referred to as t values since they should be distributed roughly as t with n degrees of freedom. In testing the significance of the t values of column E, 17 degrees of freedom, corresponding to V_D , were arbitrarily used. For 17 degrees of freedom a t value of 2.11 is significant at the 0.05 level and one of 2.90 at the 0.01 level. Only one of the t values in table 3 (test 25) is significant at the 0.05 level, and none at the 0.01 level. This result is in agreement with theory. A more sensitive test for the distribution of the individual t values is obtained from the ratio of $\Sigma(t-\bar{t})^2$ to the theoretical variance. This we shall call χ^2 since these ratios should be distributed roughly as χ^2 with $n-1$ degrees of freedom. For n degrees of freedom entering into the calculation of S_M the theoretical variance is $n/(n-2)$. Taking $n = 17$, the theoretical variance is 1.133, $\Sigma(t-\bar{t})^2 = 24.6567$, and $\chi^2 = 24.6567/1.133 = 21.76$ giving odds greater than .5 but less than .7 that the distribution of t values in column E is due entirely to chance. The mean of the t values of column E is -0.184 and the standard deviation of t_m is 0.194. The mean therefore does not differ significantly from 0, indicating that there was no significant bias in the estimation of the relative activity of the unknown.

DISCUSSION.—There are one or two further points of interest in the data of table 3. First, in only three of the 26 tests (Nos. 1, 6, 15) did the dilution curves for S and for U show a significant departure from parallelism. Second, as indicated in column G of table 3, in no case did the true slope of the com-

bined dilution curve equal or exceed unity. Its average value was approximately 0.65. It is apparent therefore that the calculation of the activity of an unknown with an assumed slope of unity would have resulted in a positive bias when the activity of the unknown was less than that of the standard, or in a negative bias when the unknown was more active than the standard. Third, comparison of the results of tests 1 to 9 with those of 14 to 25 indicates that the accuracy was considerably greater when U was 50 per cent of S than when it was only 25 per cent of S . This finding was expected and agrees with that observed previously in the measurement of tobacco-mosaic virus (Spencer and Price, 1943). The results when U was 35 per cent of S were even more accurate than when U was 50 per cent of S , but this comparison is of doubtful significance because of the small number of tests involved with $U = 0.35 S$. The comparison suggests that the accuracy of measuring the activity of an unknown can be increased by making several experiments, adjusting the concentration of the standard on the basis of the finding for the early experiments before proceeding with the later ones.

It has been pointed out by Bartlett (1936) that with the Poisson series the square root transformation leads to the variance being more nearly equal at all levels of response. The square root transformation applied to the data of the 26 experiments here considered led to no better estimates of the activities of the unknown nor to more uniform standard errors. Moreover, many of the tests showed a significant departure from parallelism of the curves for the standard and unknown. This lack of parallelism seems sufficient grounds for rejecting the square root transformation in preference to the log transformation.

In attempting to generalize from the specific experimental results here obtained, one essential fact should be kept in mind. The present tests were carried out under more or less ideal conditions; the activities of the unknowns, as well as those of the standards, were sufficiently well known that proper dilutions for the most accurate results were used. In practical application of the method the dilution factor which is chosen may not always lie within the ideal range and the calculated estimate of activity may thus be in error by a greater amount than it would have been under more ideal conditions. However, this increased error will probably be reflected in the standard error of estimate so that the limits calculated from the standard error will, in the desired proportion of the tests, include the true value of the unknown. If a narrower range is desired, this can be provided by carrying out additional tests.

SUMMARY

This paper discusses a method for measuring the activity of southern bean mosaic virus (*Marmor laesiofaciens* Zaum. and Harter), which depends upon the comparison of the local lesions produced

in Early Golden Cluster bean plants by two dilutions of a virus preparation designated as a standard with those produced by two dilutions of a preparation called an unknown. There are presented several dilution curves of the virus to show why the assumption of a slope of unity leads to error in estimation of virus activity, and therefore why two or more dilutions must be employed to determine the slope in each experiment.

The data show that the activity of the virus can be measured with an error that seldom exceeds 10 or 15 per cent, when the proper concentration of the unknown and the standard are chosen for the test. Moreover, it was shown that in experiments carried out by the method described the probability is 0.95 that the estimate will not differ from the true

value by more than 40 per cent on the one hand, nor by more than 25 per cent on the other, when dilutions are chosen such that the unknown is about 25 per cent of the standard in the first case or about 50 per cent in the second. The data also show that the accuracy is apt to be greater when the concentrations of the unknown and the standard used in the tests differ by only 50 per cent than when they differ by 75 per cent. Finally, the data show that the error of measurement is indicated fairly accurately by the standard error of the estimate, which may be calculated from the data.

DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY,
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY

LITERATURE CITED

- BALD, J. G. 1937a. The use of numbers of infections for comparing the concentration of plant virus suspensions. I. Dilution experiments with purified suspensions. *Annals Appl. Biol.* 24: 33-55.
- . 1937b. The use of numbers of infections for comparing the concentration of plant virus suspensions. II. Distortion of the dilution series. *Annals Appl. Biol.* 24: 56-76.
- . 1937c. The use of numbers of infections for comparing the concentration of plant virus suspensions. IV. Modification of the simple dilution equation. *Australian Jour. Exp. Biol. and Med. Sci.* 15: 211-220.
- BARTLETT, M. S. 1936. The square root transformation in analysis of variance. *Suppl. Jour. Roy. Stat. Soc.* 3: 68-78.
- BLISS, C. I., AND H. P. MARKS. 1939a. The biological assay of insulin. I. Some general considerations directed to increasing the precision of the curve relating dosage and graded response. *Quart. Jour. Pharm. and Pharmacol.* 12: 82-110.
- , AND ———. 1939b. The biological assay of insulin. II. The estimation of drug potency from a graded response. *Quart. Jour. Pharm. and Pharmacol.* 12: 182-205.
- SPENCER, ERNEST L., AND W. C. PRICE. 1943. Accuracy of the local-lesion method for measuring virus activity. I. Tobacco-mosaic virus. *Amer. Jour. Bot.* 30: 280-290.
- PRICE, W. C., AND ERNEST L. SPENCER. 1943a. Accuracy of the local-lesion method for measuring virus activity. II. Tobacco-necrosis, alfalfa-mosaic, and tobacco-ringspot viruses. *Amer. Jour. Bot.* 30: 340-346.
- , AND ———. 1943b. Accuracy of the local-lesion method for measuring virus activity. III. The standard deviation of the log-ratio of potencies as a measure of the accuracy of measurement. *Amer. Jour. Bot.* 30: 720-735.
- YODEN, W. J., HELEN PURDY BEALE, AND JOHN D. GUTHRIE. 1935. Relation of virus concentration to the number of lesions produced. *Contrib. Boyce Thompson Inst.* 7: 37-53.
- ZAUMEYER, W. J., AND L. L. HARTER. 1942. A new virus disease of bean. *Phytopathology* 32: 438-439.
- , AND ———. 1943. Two new virus diseases of beans. *Jour. Agric. Res.* 67: 305-328.
- , AND ———. 1944. A severe necrosis caused by bean-mosaic virus 4 on beans. *Phytopathology* 34: 510-512.

RUBBER IN CRYPTOSTEGIA LEAF CHLORENCHYMA¹

Robert T. Whittenberger and Albert Kelner²

ALTHOUGH RUBBER is a fairly common constituent of higher plants, most information concerning its origin, function, and anatomical occurrence has been obtained from investigation of *Hevea brasiliensis* Muell. Arg., or in more recent years, of *Parthenium argentatum* Gray and *Taraxacum kok-saghyz* Rodin (Lloyd, 1911, 1932; Bobilioff, 1923; Memmler, 1934; Spence and McCallum, 1935; Mazanko, 1938, 1940; Prokof'ev, 1939, 1940). Rubber in the dis-

persed or latex form occurs in laticiferous ducts in *Hevea* and *Taraxacum*, and in parenchyma and resin canal cells in *Parthenium*. The modes of rubber distribution in these plants have been accepted as prototypes. The possibility that rubber may occur also in regions other than those mentioned, that non-latex as well as latex rubber may occur in the same plant, and that there may be some relation between rubber and such structures as chloroplasts has been overlooked.

With the wartime loss of *Hevea* rubber, other plants have been considered as sources of natural rubber. One of these, *Cryptostegia*, has abundant latex, and a well-developed system of laticiferous ducts ramifies throughout the entire plant (Dolley, 1911; Polhamus *et al.*, 1934; Viswanath *et al.*,

¹ Received for publication June 22, 1945.

Natural Rubber from Domestic Sources. Paper No. 9.

² Formerly of this Laboratory; now at the University of Pennsylvania, Philadelphia, Pa.

The chemical analyses, which required specially developed micro-techniques, were performed by C. O. Willits and C. L. Ogg. The assistance and cooperation of other members of this laboratory are also gratefully acknowledged.

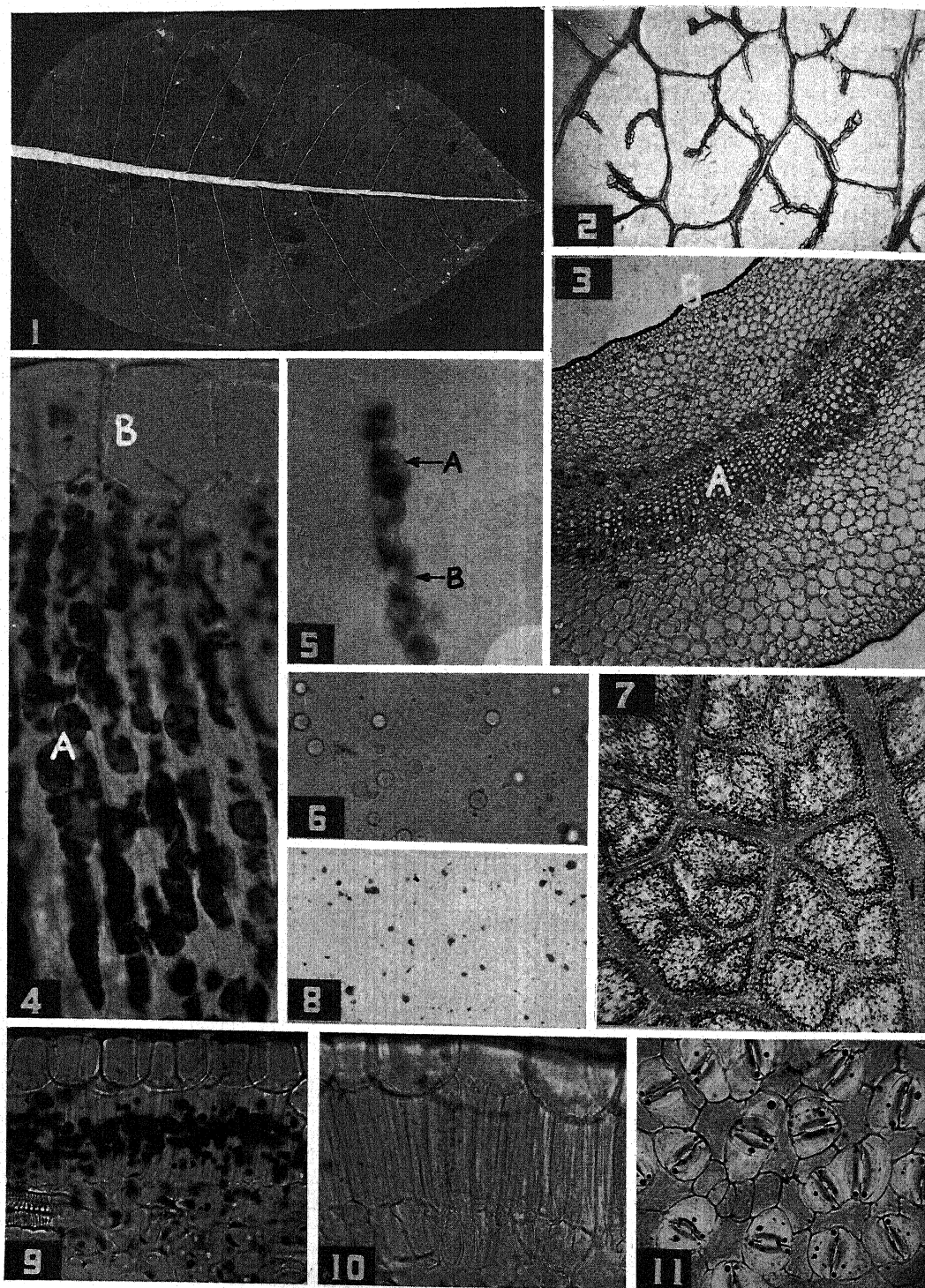


Fig. 1-11. Photomicrographs of *Cryptostegia* hybrid leaf.—Fig. 1. Vein pattern, $\times 1$, obtained by retting the leaf and removing all tissues except the xylem.—Fig. 2. Xylem of small veins of retted leaf. $\times 50$.—Fig. 3. Transverse section of midrib, acetone extracted. $\times 50$. Stained areas above and below the xylem (A) represent rubber in laticiferous ducts. B, upper epidermis.—Fig. 4. Transverse section of fresh, living, mature leaf, mounted in water, untreated, unstained. $\times 460$. Rubber-bearing globules (A) intermingled with the darker, smaller chloroplasts in the palisade cells are shown. B, upper epidermis.—Fig. 5. Unstained, naked, retted palisade protoplast showing several of the globules. $\times 940$. A, single globule; B, chloroplast.—Fig. 6. Rubber-bearing globules isolated from the leaf chlorenchyma. $\times 460$. Some globules are not in focus.—Fig. 7. Peridermal section of spongy mesophyll showing numerous

1943; Blaser, 1945). Heretofore it has been assumed that all the rubber of this plant occurred as latex in the duct system. However, during a study of the latex system of the leaves, it was noted that chlorenchyma cells contained large globules which stained intensely with oil-soluble stains such as Sudan III and oil blue NA (Whittenberger, 1944) but were like rubber in that they were insoluble in acetone and nonsaponifiable.

Because of the theoretical and practical importance of a possible relation between rubber and chlorophyll in this plant, an intensive study of these globules was initiated. Most investigations on the origin of rubber in plants having laticiferous ducts have centered exclusively upon the ducts as the site of rubber formation (Bobiloff, 1923; Mann, 1932; Mazanko, 1938; Blokhintseva, 1940; Prokof'ev, 1944; and others). Memmler (1934) stated that it had not yet been possible to detect rubber in other cells, especially in the parenchyma cells surrounding the laticiferous elements. Furthermore, previous workers on *Cryptostegia* (Dolley, 1911; Polhamus *et al.*, 1934; Viswanath *et al.*, 1943; Blaser, 1945) did not mention the presence of rubber in the leaf chlorenchyma.

MATERIAL AND METHODS.—The principal observations were made on leaves of *Cryptostegia* hybrid (*C. madagascariensis* Boj. \times *C. grandiflora* R. Br.) and *Cryptostegia grandiflora* R. Br. Most of the plants were grown in Florida and shipped under refrigeration to Philadelphia at frequent intervals; other plants were sent from Cuba and Mexico. Supplemental observations were also made on fresh greenhouse plants.

Leaves were sectioned free hand or with a sliding microtome and examined either immediately in a drop of water, or after staining with Sudan III or oil blue NA. Although the globules could be seen in living, unstained leaf sections, they were most clearly brought out by the following procedure. Sections were treated with Javelle water for ten minutes, after which they were thoroughly washed in water, rinsed in 50 per cent ethanol, and immersed for 20 minutes in a saturated solution of oil blue NA in 50 per cent ethanol. They were then rinsed briefly in 50 per cent ethanol and in water and were examined either in water, glycerin, Karo, or glycerin jelly. Many observations, especially on the laticiferous ducts, were made with the dissecting microscope in order to avoid the displacement and loss of rubber attendant on the preparation of sections. Procedures for determining solubility and other characteristics are described later.

RESULTS.—*Distribution of latex ducts in the leaf.*—The laticiferous ducts are present in approxi-

mately equal numbers in the tissue above and below the leaf veins which they follow throughout the blade. Typical vein patterns are shown in figures 1 and 2, and a cross section of the midrib in figure 3. In a mature leaf, the ducts are most numerous (50 to 100) and largest (10 to 25 microns in diameter) along the midrib; they decrease gradually in number and size as the veins branch. Associated with each of the 20 to 25 largest lateral veins are 10 to 20 ducts of about 8 to 20 microns in diameter. Some ducts come in contact with the palisade parenchyma and epidermis (Blaser, 1945). Each of the smallest veins terminates with a single tracheid (fig. 2) and occasionally is devoid of ducts. Individual leaves differ widely in the number and size of ducts, and probably in the amount of latex rubber they contain.

Distribution of leaf globules.—The globules are clearly visible as colorless, hyaline, homogeneous masses even in unstained sections of living mature leaves (fig. 4). Figure 5 shows the globules in a retted palisade protoplast, and in figure 6 they are shown completely isolated. Frequently the larger globules are partly surrounded by chloroplasts. The globules occur in the cytoplasm of all the green mesophyll cells, although they are most numerous (1 to 8 per cell) in the palisade layer. In this layer they are uniformly distributed and of equal concentration throughout the leaf blade. In the dorsal half of the leaf (spongy parenchyma), the globule-bearing cells are more numerous adjoining the ducts than in nonduct areas (fig. 7).

The globules may attain a diameter of 10 to 12 microns (in the palisade cells) although smaller diameters (3 to 7 microns) are more common. They are therefore considerably larger than the rubber particles (fig. 8) of the leaf laticiferous duct system. It should be emphasized, however, that the globules vary greatly in size, frequency, and shape, depending upon the development, age, and previous history of the leaf. In young leaves there are only small globules, or none at all; in mature leaves of unusually high rubber content, the chlorenchyma cells are heavily laden with globular material. In the latter cells, many of the globules are distended to form oval, sheet-like, or rod-shaped masses. A rapid estimate of the quantity of rubber in a leaf may be made by noting microscopically the extent of the globular material of the cell.

Character of the unextracted leaf globules.—There are no accurate, direct, microchemical tests for determining rubber in small plant cells. Oil blue NA and other oil-soluble stains do not differentiate rubber from lipids, cutin, resins, suberin, and various waxes, and solubility tests alone are inconclusive. For preliminary determinations, however, the

rubber-bearing globules and channels of lower epidermis exposed by the removal of the veins. Bleached with Javelle water, stained with oil blue NA. $\times 50$.—Fig. 8. Electron micrograph of latex from the leaf laticiferous duct system. $\times 1280$.—Fig. 9. Transverse section, bleached with Javelle water, acetone-extracted for 48 hours, and stained with oil blue NA. $\times 215$. Stained bodies in the chlorenchyma are rubber.—Fig. 10. Transverse section, bleached with Javelle water, ether-extracted for 24 hours, and stained with oil blue NA. $\times 200$. No rubber remained after ether extraction.—Fig. 11. Lower epidermis showing globules in the guard cells. Bleached with Javelle water, stained with oil blue NA. $\times 215$.

collective use of staining and solubility tests consistently indicated that the globules contained rubber.

Stains such as oil blue NA, Sudan III, Sudan IV, oil red N-1700, or alkanet applied directly to the leaf stained these globules intensely, sharply differentiating the larger ones from the chloroplasts, although some of the smaller globules were obscured. The globules could be more distinctly demonstrated if the cells were first cleared and bleached with Javelle water or sodium hypochlorite solution. Upon treatment with Javelle water, the chloroplasts and even the protoplasts disintegrated and disappeared, whereas the large globules remained essentially unchanged. As certain chloroplasts disintegrated, small bright globules, which previously could not be observed, appeared in their places. These were apparently identical in nature with the original globules in the intact cell, and took the rubber stains in the same manner. It was uncertain whether these new globules were originally distinct but hidden by the chloroplasts, or whether they were formed from the disintegrating chloroplastic material.

Solubility of globules was determined by putting sections into absolute ethanol or acetone and then into particular solvents, in which they were kept at room temperature for 24 to 48 hours, or at boiling temperature for 8 to 16 hours. They were then again put into acetone or ethanol, washed with water, treated with Javelle water, washed with water again and then with 50 per cent ethanol, stained, and examined. Usually, however, it was more convenient to treat sections with Javelle water first and, after extraction, to examine the sections directly in acetone or ethanol without staining. Control experiments showed that treatment with Javelle water did not alter the solubility of the globules. Solvents of relatively high polarity were applied directly to untreated sections and were permitted to act from two to four hours.

Table 1 shows that the globules had the characteristics of rubber. That they were not composed largely of resins was indicated by their insolubility in ethanol, acetone, sulfuric acid, and chloral hydrate; that they were not waxes or fat globules was suggested by their insolubility in acetone (fig. 9) and their nonsaponifiability. The globules were distinguished from gums and mucilages by being insoluble in boiling water, chloral hydrate, and Javelle water, and soluble in ether (fig. 10). Moreover, they dissolved in the common rubber solvents, such as benzene, xylene, and carbon tetrachloride.

The behavior of the globules in acetone and ether was characteristic enough to merit particular discussion. Acetone caused some of the globules to lose their characteristic globular morphology, shrink somewhat, and stick in patches to the cell wall (fig. 9). These bodies stained as brilliantly as ever with oil blue NA. Drastic acetone extraction carried out for 32 hours at 25°C. and for 16 hours at boiling temperature in no way altered this picture.

TABLE 1. *Effect of various treatments on globules in Cryptostegia hybrid leaf.*

Treatment	Observable effect on globules
Distilled water	None
95% ethyl alcohol	None
Absolute alcohol	None
Methyl alcohol	None
Acetone	Shrink moderately, some stick to cell wall (see text)
Ethyl ether	Dissolve (see text)
Benzene	Dissolve (see text)
Xylene	Dissolve
Carbon tetrachloride	Dissolve
Carbon disulfide	Dissolve
Chloroform	Dissolve imperfectly
Glacial acetic acid	Appear finely pitted
10% potassium hydroxide in 95% ethanol	Shrink slightly, but are not saponified
Potassium hydroxide-ammonia reagent	Shrink slightly, but are not saponified
Aqueous chloral hydrate	None
5% sulfuric acid	None
72% sulfuric acid	Swell slightly, but remain homogeneous
Javelle water	Appear finely pitted
10% ferric chloride	Negative, tannin absent

Since Spence and Caldwell (1933) have shown that sometimes fats are rendered acetone-insoluble by admixture with proteins, sections were treated in the following manner to hydrolyze and remove proteins before acetone extraction:

- (1) Water extraction for 16 hrs. at 25°C., + 8 hrs. boiling.
- (2) 5 per cent H_2SO_4 extraction for 16 hrs. at 25°C., + 8 hrs. boiling.
- (3) Water extraction for 16 hrs. at 25°C., + 8 hrs. boiling.
- (4) Acetone extraction for 32 hrs. at 25°C., + 16 hrs. boiling.

After step 4, staining of sections revealed that globules with characteristic "acetone" morphology were still present. Finally, after step 4, sections were extracted with benzene for 32 hours at 25°C., plus 16 hours at the boiling temperature. No globules were present in the somewhat macerated sections.

When sections were extracted in ether or benzene for short periods, 18 hours or less at 25°C., another characteristic picture developed. In one experiment, for example, sections were treated with Javelle water, then ether-extracted for 18 hours at 25°C. The cells appeared entirely empty when examined in ether (fig. 10). These sections were then transferred directly to a drop of absolute acetone on the slide and examined. The cells became filled with myriad tiny particles in active Brownian motion, which stained brilliantly with oil blue NA. In another experiment in which sections were given the same treatment except that they were ether-extracted for 48 hours, the addition of acetone

caused no formation of colloidal particles. It was probable that ether treatment for 18 hours at 25°C. dissolved the globules, perhaps filling the cell with a viscous solution which did not penetrate the cell wall readily. Subsequent addition of acetone precipitated the globules in colloidal form within the cell. These colloidal particles redissolved when ether was added, but were reprecipitated by acetone. Since the refractive index of acetone, 1.35, is almost identical with that of ether, 1.36, it is apparent that the phenomenon cannot be explained by a difference in refractive index of the two solvents.

A similar experiment carried out with benzene instead of ether was less satisfactory because the entire section was quite transparent. However, a fine suspension appeared within the cells when the sections were treated with acetone. It should be emphasized that this phenomenon appeared only in fairly thick sections (50 to 100 μ) extracted for 18 hours or less in the solvent at room temperature. Longer extraction, or heat, rid the cell of all ether- or benzene-soluble material, and no precipitation by acetone occurred.

Further information was obtained by micrurgical tests. Globules were obtained free of cell walls by mechanical disintegration of leaves softened with dilute alkali. When a single globule was subjected to tension by two micro-needles, it stretched about tenfold before rupture. After rupture, the stretched threads quickly retracted, and two separate globules were formed. The globule, however, was tacky and when punctured stuck to the glass needle, suggesting the presence of rubber of low molecular weight. These staining, solubility, and micrurgical tests strongly indicated, although they did not prove, that the chlorenchyma globules contained rubber.

Chemical analysis of dissected leaf fractions.—Chemical analysis of an entire *Cryptostegia* hybrid leaf showed that it contained about 3 to 5 per cent of rubber on the dry-weight basis. It was difficult to determine what portion of this rubber was due to the latex in the leaf, and what portion, if any, was contributed by the globules in the chlorenchyma. Evidence on this point could be obtained by separate chemical analysis of the latex-bearing and nonlatex-bearing portion of the leaf. However, obtaining chlorenchyma cells free of latex ducts was

a task soon abandoned because of the difficulty of isolating them in quantity sufficiently large for chemical analysis. Rather, the following experiment was carried out.

From each of six *Cryptostegia* hybrid twigs three leaves were plucked, and the sample was divided into three lots, each of which contained only one leaf from any given twig. Only uninjured, non-chlorotic, turgid, clean leaves were selected. The leaves were severed at the abscission layer, and no latex was observed to have been lost through exudation. They were infiltrated under suction successively with dilute acetic acid, water, and dilute sodium hydroxide in order to coagulate any dispersed latex and to soften them sufficiently for dissection.

Since previous anatomical studies had indicated that the quantity of latex duct rubber was closely correlated with the quantity of veins, it was believed that removal of the main vein would remove a like proportion of the latex ducts. In a separate experiment on retted leaves (see fig. 1 and 2 for vein pattern), it was shown that the main vein and petiole contained by weight more than half the xylem of all the veins of the leaf, and that the xylem of the 20 to 25 largest lateral veins contributed only a small fraction of the total weight. Accordingly, two of the three lots of leaves were dissected with micro-scalpels under the stereoscopic microscope. The first lot was separated into two fractions; 1a comprised the petiole and midrib with its associated ducts, and 1b comprised the remainder of the leaf tissues. The second lot was divided into three fractions: 2a was identical with 1a; 2b comprised the 24 largest lateral veins and associated ducts of each leaf; and 2c comprised the remainder of the leaf tissues, including the smallest veins and ducts. The third lot was not dissected and served as a control. Since the globule-bearing cells are most numerous near the veins and ducts (fig. 7), extreme care was used in all cases in separating these cells from the vein-duct fraction. All fractions were analyzed for total rubber, with the results shown in table 2.

The unusually high figures for the benzene extract were undoubtedly due to the removal from the leaves of some of the nonrubber plant constituents by the acid, water, and alkali treatment before dissection. However, as shown in the last column, the

TABLE 2. Analysis of dissected *Cryptostegia* hybrid leaf fraction.

Leaf fraction	Weight of leaf fraction, g. ^a	Resin (acetone extract), per cent ^a	Rubber (benzene extract), per cent ^a	Per cent of total leaf rubber
1(a) Petiole and midrib	0.177	7.5	6.6	9.0
1(b) Remaining leaf tissue	1.248	14.7	9.5	91.0
2(a) Petiole and midrib	0.157	8.9	4.3	5.3
2(b) Largest lateral veins	0.038	13.9	5.0	1.5
2(c) Remaining leaf tissue	0.934	15.8	12.5	93.2
3 Undissected leaf	1.061	15.4	9.7	100

^a Moisture-free basis.

distribution of rubber was not affected by this pretreatment. It is concluded that 85 to 90 per cent of the total rubber of these leaves occurred in chlorenchyma cells, that only 10 to 15 per cent existed in the latex ducts, that the ducts of the largest lateral veins contained only an insignificant fraction of the total rubber, and that most of the latex rubber in the leaves occurred in the petiole and midrib. Furthermore, on the basis of the data above and the chemical analysis of plant organs for rubber (Viswanath *et al.*, 1943), it appears that the leaf chlorenchyma globules contain the greater portion of the total rubber in the average three or four year old plant, the smaller portion occurring as latex in the stem, leaf, and root.

To estimate that 85 to 90 per cent of the total leaf rubber occurred in chlorenchyma cells, it was necessary to assume that latex rubber was no more concentrated in the small ducts in the undissected portions of the blade than in the dissected duct fractions. Microscopic examination indicated that the latex rubber was actually more concentrated in the larger ducts associated with the larger veins. In fact, some of the smallest ducts of the smallest veins appeared to contain structureless material similar to that of the globules rather than typical latex. It has already been pointed out that on the average the ducts are largest and most numerous along the largest veins. It seemed justified, therefore, on the basis of the total amount of leaf rubber in the ducts of the petiole and midrib (average 7.2 per cent) and in the 24 largest lateral veins (1.5 per cent), to assume that even less than 7.2 per cent of the total rubber remained in the small, unseparated ducts of the blade. The estimate that 85 to 90 per cent of the total rubber occurred in the leaf chlorenchyma receives independent support from data obtained during research on the recovery of rubber from *Cryptostegia* (Whittenberger, Brice and Copley, 1945).

The microscopic observation that individual leaves vary widely in the amount of latex rubber they contain is substantiated by the data in table 2. For example, the latex ducts associated with the main vein and petiole of lot 1 contained 9.0 per cent of the total leaf rubber, whereas those of lot 2 contained only 5.3 per cent of the total. The main vein and petiole fraction of lot 1 also contained rubber in greater concentration (6.6 per cent) and resin in less concentration (7.5 per cent) than did the corresponding fraction of lot 2 (4.3 per cent rubber and 8.9 per cent resin). These facts suggested that the average age of lot 1 was greater than that of lot 2, since the ratio of rubber to resin and oil becomes greater as the plant grows older (Prokof'ev, 1939; Blokhintseva, 1940; Moshkima, 1940).

Properties and composition of isolated globules.—Although data indicated that the bulk of the rubber occurred in leaf cells exterior to the laticiferous duct system, they were not entirely conclusive. The possibility existed that the chemical method of analysis (based upon the fact that rubber is soluble

in benzene and insoluble in acetone) was incapable of distinguishing rubber from another substance or combination of substances which possessed certain properties similar to those of rubber. Moreover, since the leaf was not completely dissected, exactly how much latex rubber was mixed with the cell rubber was not determined. Conclusive proof that the leaf chlorenchyma globules contained rubber could be obtained by isolating the globules in quantity sufficient for physical tests and x-ray analysis. With the development of a method for the recovery of rubber from *Cryptostegia* leaves by Naghski and associates (1945), a means of isolation of the globules became available. During the isolation special precautions were taken to assure that the separation of the cell globules from the latex rubber was complete. The effect of each step of the process on the globules and latex was followed microscopically. The isolated mass of chlorenchyma globules was yellowish, soft, tacky, and elastic, and exhibited noticeable snap when stretched and released. The results of chemical analysis of the globules are shown in table 3.

TABLE 3. Chemical analysis of fresh mature *Cryptostegia* hybrid leaves (starting material) and the globules isolated from the leaf cells.

	Original leaves	Isolated cell globules
Wet weight, grams.....	3,350	11.4
Dry weight, grams.....	450	8.7
Moisture, per cent.....	86.5	24.3
Acetone solubles by direct extraction, per cent ^a	9.4	29.6
Rubber hydrocarbon in acetone solu- bles, per cent ^a	3.8
Acetone solubles, nonrubber, per cent ^a	25.8
Acetone and benzene insolubles, per cent ^a	1.3
Total rubber hydrocarbon, per cent ^a ...	3.5	65.0
Nonrubber benzene-soluble acetone-in- solubles, by difference, per cent ^a	7.9

^a Moisture-free basis.

About 65 per cent of the globular material was rubber. About 8 per cent of the isolated globules was nonrubber, benzene-soluble, acetone-insoluble material of unknown composition. Rubber hydrocarbon in the acetone extract, as indicated by the analysis, might be expected if rubber of low molecular weight is present in the globules. It was determined gravimetrically by precipitating the hydrocarbon as rubber "tetrabromide" and establishing the identity of the precipitate by bromide analysis. Hoover *et al.* (1945) found that 93 per cent of the globular material was soluble in methyl ethyl ketone, an indication of the low degree of polymerization of the rubber (Cheyney, 1942). Spectrophotometric examination of the benzene solution of the globules indicated the presence of pheophytin and carotene.

Another portion of the globular material, after being compounded by a modified A.C.S. formula containing 50 parts of channel black, was vulcanized and tested for rubberlike properties. The properties of the vulcanizate—ultimate elongation, modulus at 300 per cent elongation, and tensile strength—were characteristic of rubber of low molecular weight. Later and more detailed information on the character of this rubber has been published by Hoover *et al.* (1945).

Final proof of the existence of rubber in the isolated cell globules was furnished by x-ray diffraction studies of the vulcanizate. At 320 per cent elongation, slight arcing of the innermost amorphous ring became evident. At 450 per cent elongation, eight crystalline reflections became visible. The interplanar spacings determined from these discrete reflections agreed with those of stretched *Hevea* rubber, indicating the presence of a *cis*-polyisoprene molecular structure.

Discussion.—The chlorenchyma rubber in *Cryptostegia* leaf represents a hitherto unrecognized mode of rubber distribution and storage in this plant. Whether leaf chlorenchyma rubber is as widely distributed among plants as is latex rubber, or whether other plants exist in which both laticiferous duct and leaf chlorenchyma rubber occur simultaneously, is not known. Naylor (1943) found rubber both in ducts (long rod-like cells) and in the mesophyll parenchyma of *Eucommia ulmoides* Oliver. Little detail is given, however, as to the method by which he determined that the globular masses in the parenchyma were rubber. Novikov *et al.* (1934) stated that in *Scorzonera tau-saghyz* Lips. & Bos. and in *Apocynum venetum* L. rubber may be detected in the green parenchyma of the leaf notwithstanding the latex cells in the leaf. Kiselev *et al.* (1934) also reported that rubber is formed in the photosynthetic tissue of *Scorzonera tau-saghyz* Lips. & Bos. and *Chondrilla* spp., and passes from there in an unknown form into the latex ducts. These observations, however, have not been generally accepted and are at variance with the findings of Prokof'ev (1939, 1944).

Although Lloyd (1911) reported finding rubberlike bodies in the leaf chlorenchyma of *Parthenium argentatum* Gray, subsequent workers (Artschwager, 1943; Moshkima, 1940) do not mention similar bodies in the leaves of this plant. The rubber occurring in the stem chlorenchyma of *Parthenium* (Spence, 1928, 1938; Lloyd, 1932) is a latex markedly different from the nonlatex rubber reported here, and probably bears a different relationship to chlorophyll. In *Chrysothamnus* spp. Hall and Goodspeed (1919) reported that one or more rubber globules may be detected in each of the palisade cells. There are no laticiferous ducts in this plant. Little detailed information is available concerning the anatomical disposition of rubber in *Solidago* spp. other than that it occurs principally in the leaves (Polhamus, 1933) and apparently is localized in parenchymatous tissues and in certain

isolated areas (Legros, 1937). Our preliminary studies of *Solidago* spp. showed acetone-insoluble, rubberlike globules in the palisade and spongy mesophyll cells. These globules appeared similar to those in *Cryptostegia* leaf. However, no laticiferous duct rubber was observed.³

Our anatomical studies on the leaves of *Taraxacum kok-saghyz* Rodin revealed oil or resinlike globules in the chlorenchyma. These globules, accordingly, differed from those in *Cryptostegia* leaf in that they contained no rubber and were soluble in ethanol and acetone. All the leaf rubber was found in the laticiferous ducts. The possibility, however, that at least some of the latex rubber is synthesized in the leaf ducts is not denied by these observations. Therefore, they do not necessarily support the conclusion of Blokhintseva (1940) that the rubber of this plant is synthesized in the latex vessels of the root and that the synthesis bears no direct connection with the assimilating organs.

For obvious reasons, demonstration of leaf chlorenchyma rubber, especially in the presence of latex rubber as in *Cryptostegia*, was less simple than the demonstration of latex rubber alone. While all available staining and microchemical tests indicated that the globules contained rubber, final proof was obtained by x-ray analysis, which showed the *cis*-polyisoprene molecular structure. Confirmatory evidence was obtained during experimentation on methods for the large-scale recovery of rubber from the leaves (Naghski *et al.*, 1945; Whittenberger *et al.*, 1945).

The association of rubber with chloroplasts raises the question of the relationship between the food-manufacturing organs and chlorenchyma rubber. Since young leaves lack globules, and globules form and enlarge as the leaves mature, it is probable that the rubber is accumulated slowly within the cells and requires an excess of photosynthetic products. Thus the palisade cells with their abundance of chloroplasts probably accumulate a greater excess of photosynthetic products, and hence rubber, than the spongy mesophyll cells. The apparent increase in the number of rubber-bearing globules brought about by the disintegration of chloroplasts by Javelle water suggests the possibility that some rubber is formed within the chloroplast itself, in a manner analogous to starch formation within chloroplasts or oil formation within elaioplasts. Prokof'ev (1939) and Blokhintseva (1940) are of the opinion that the synthesis of latex rubber in *Taraxacum kok-saghyz* Rodin probably occurs in the plastids of the laticiferous ducts.

There can be little doubt that the chlorenchyma rubber originates in the leaf. This rubber appears in no other part of the plant. There is a question, however, concerning the origin of the widely dis-

³ Recently M. E. Rollins, T. L. W. Bailey, Jr., and I. V. deGruy (unpublished report at the Southern Regional Research Laboratory, New Orleans, La., 1945) have shown that the rubber in *Solidago leavenworthii* Torr. & Gray occurs as small globules, 1 to 5 per cell, in the spongy mesophyll and palisade cells of the leaf.

tributed latex rubber. Viswanath *et al.* (1943), on the basis of studies on the quantity and composition of latex before and after the defoliation period, suggest that the latex rubber of *Cryptostegia grandiflora* R. Br. originates in the organs of photosynthesis. If this suggestion is accepted, then it is permissible, on the basis of our present knowledge of the character and disposition of the chlorenchyma globules, to speculate on a possible relation between the globules and the duct rubber. The investigators named above evidently were unaware of the chlorenchyma globules. It is conceivable that the globule rubber is deposited in chlorenchyma cells by a mechanism analogous to that which causes the accumulation of starch in leaf chlorenchyma of many plants. This relatively unstable rubber might then be digested to such a state that its translocation to the ducts would be possible. Within the ducts synthesis of large rubber molecules could occur, resulting in the formation of latex rubber of good quality. Translocation and synthesis could occur at a substantially continuous and rapid rate, constantly replenishing the latex rubber as it is withdrawn from the plant every one to three days (Symontowne, 1943; Fennell, 1944). Such a conception would account for the observed diminution in latex flow upon defoliation, and is consistent with the observation that the degree of polymerization of rubber of the leaf ducts apparently is higher than that of the chlorenchyma cells (Hoover *et al.*, 1945). Micrurgical evidence indicates that the degree of polymerization of the leaf duct rubber is intermediate between that of the stem ducts and chlorenchyma cells.

In this conception, an early precursor of latex rubber, that is, the material translocated from the cells to the ducts, would presumably consist of a compound less oxidized than simple sugars.⁴ Mann (1932) stated that no simple sugars have been isolated from the latex of *Hevea brasiliensis* Muell. Arg., although he held the opinion that the latex rubber was synthesized directly from temporary simple sugars in the ducts.

While *Cryptostegia* may be outstanding in possessing leaves that contain both laticiferous duct and chlorenchyma rubber, it is not unique in having the major portion of its leaf rubber low in molecular weight. There is evidence also that the leaf rubber of *Asclepias syriaca* L. (Paul, Blakers, and Watson, 1943) and *Solidago leavenworthii* Torr. & Gray (Skau *et al.*, 1945) is of low molecular weight. Such a condition might be expected if it is admitted that a large portion of material synthesized in the leaf, whether rubber or otherwise, is rapidly translocated, in simple form and by some as yet unknown mechanism, to the more permanent parts of the plant.

⁴ There is evidence that the precursor complex may include lupeol esterified with two hydroxy-n-fatty acids (unpublished report by S. G. Wildman, S. B. Hendricks, F. A. Abegg, J. A. Elder, and P. E. Heath of the Bur. Plant Ind., Soils and Agric. Eng. at Beltsville, Maryland, 1945).

In view of the close relationship between *Cryptostegia* chlorenchyma rubber and photosynthetic activity of the cell it is difficult to see how this rubber can be merely a waste product or a wound-healing agent, or serve in many of the other roles commonly suggested for latex rubber. It is far more likely that this rubber is a storage product of definite physiological significance. Whether it represents a highly efficient form of food storage, whether its importance lies in its ability to remove osmotically active simple sugars from the actively photosynthesizing cell, whether it is the precursor of latex rubber, and what factors determine its degree of polymerization are questions which must await physiological experimentation.

An interesting corollary of the observation that rubber and chlorophyll co-exist in the same cell in *Cryptostegia* leaf mesophyll is the absence of similar globules in the guard cells of the lower epidermis. Although globules are clearly visible in the guard cells of both fresh and Javelle water-treated strips of the epidermis (fig. 11), tests on these globules indicate that they are different from those of the mesophyll chlorenchyma, as only a few, if any, of them contain rubber. For the most part they are dissolved by ethanol or acetone and are removed by alcoholic potassium hydroxide. These observations, therefore, indirectly support the conclusion of Sayre (1926) that the green pigment of the guard cells is not identical with the chlorophyll of the leaf mesophyll. Otherwise, it might be expected that rubber globules similar to those in the mesophyll would occur also in the guard cells, since the globules in the mesophyll are correlated with the presence of chlorophyll.

SUMMARY

An unusual type of rubber storage has been found in *Cryptostegia* leaf. Although *Cryptostegia* possesses abundant rubber latex and a laticiferous duct system well developed in all parts of the plant, the major portion of the leaf rubber occurs as non-latex globules in the mesophyll chlorenchyma, entirely distinct from the duct system. These globules are strikingly correlated with the presence of chlorophyll, being largest (up to 12 μ in diameter) and most numerous (as many as 8 per cell) in the palisade cells of fully mature leaves. The physiological significance of the association of the globules with chlorophyll is not yet known.

In mature *Cryptostegia* hybrid leaves, 85 to 90 per cent of the total rubber is in the chlorenchyma, the remaining 10 to 15 per cent occurring as latex in the laticiferous ducts. The ducts follow the veins throughout the blade and are largest and most numerous along the midrib.

Proof that the leaf chlorenchyma globules contained rubber was established by x-ray studies of the isolated globules, after exhaustive staining and microchemical tests on leaf sections. The chlorenchyma rubber, although possessing a *cis*-polyisoprene molecular structure, apparently is of lower

molecular weight than that of the laticiferous ducts. The globules contain about 65 per cent of rubber hydrocarbon, the remaining 35 per cent consisting largely of acetone-soluble material (resins).

EASTERN REGIONAL RESEARCH LABORATORY,
BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY,
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
PHILADELPHIA 18, PENNSYLVANIA

LITERATURE CITED

- ARTSCHWAGER, ERNST. 1943. Contribution to the morphology and anatomy of guayule (*Parthenium argentatum*). U.S.D.A. Tech. Bull. No. 842, 1-33.
- BLASER, H. W. 1945. Anatomy of *Cryptostegia grandiflora* with special reference to the latex system. Amer. Jour. Bot. 32:135-141.
- BLOKHINTSEVA, I. I. 1940. Formation of rubber in kok-saghyz as a result of the functioning of the latex vessels. Bull. Acad. Sci. U.R.S.S., Ser. Biol. No. 4, 608-613.
- BOBILIOFF, W. 1923. Onderzoekingen over het voorkomen van caoutchouc en melksapvaten in de bladeren van *Hevea brasiliensis*. Arch. Rubbercultuur 7:205-215.
- CHEYNEY, L. E. 1942. Methyl ethyl ketone extraction of rubber. Ind. Eng. Chem. 34:1426-1428.
- DOLLEY, C. S. 1911. On the occurrence of palay rubber (*Cryptostegia grandiflora* R. Br.) in Mexico. India Rubber Jour. 41:1156-1158.
- FENNELL, T. A. 1944. *Cryptostegia* rubber—its rebirth and development. Rubber Age 54:329-332.
- HALL, H. M., AND T. H. GOODSPEED. 1919. A rubber plant survey of western North America. Univ. California Publ. Bot. 7:239-243.
- HOOVER, S. R., T. J. DIETZ, J. NAGHSKI, AND J. W. WHITE, JR. 1945. *Cryptostegia* leaf rubber. Ind. Eng. Chem. 37:803-809.
- KISELEV, N. N., A. P. OSIPOV, AND K. A. KUZMINA. 1934. Conditions for the formation of rubber and resin and their movements in plants. Bull. Acad. Sci. U.R.S.S., classe Sci. Math. Nat. No. 9, 1367-1385.
- LEGROS, J. 1937. Secondary rubber yielding plants of the Caucasus region and of Central Asia. Internat. Rev. Agr. 28:468-481T.
- LOYD, F. E. 1911. Guayule (*Parthenium argentatum* Gray), a rubber plant of the Chihuahuan desert. Carnegie Inst. Washington Publ. No. 139:1-213.
- . 1932. Mode of occurrence of caoutchouc in the guayule *Parthenium argentatum* Gray, and its function. Plant Physiol. 7:131-138.
- MANN, C. E. T. 1932. Latex production in relation to plant physiology. India Rubber Jour. 84:653-656.
- MAZANKO, F. P. 1938. Rubber formation in tau-saghyz. Compt. Rend. Acad. Sci. U.R.S.S. 19:99-102.
- . 1940. Consumption of their rubber by certain plants. Compt. Rend. Acad. Sci. U.R.S.S. 27:838-840.
- MEMMLER, K. 1934. (Editor.) The Science of Rubber. Authorized English translation by R. F. Dunbrook and V. N. Morris. Reinhold Publishing Corp., New York.
- MOSHKIMA, M. S. 1940. Structural peculiarities of the guayule rubber plant. Bull. Acad. Sci. U.R.S.S., Ser. Biol., No. 4, 614-620.
- NAGHSKI, J., J. W. WHITE, JR., S. R. HOOVER, AND J. J. WILLAMAN. 1945. Anaerobic fermentation of *Cryptostegia* leaves for rubber recovery. Jour. Bact. 49:563-574.
- NAYLOR, E. E. 1943. Rubber from a hardy tree. N. Y. Bot. Gar. Jour. 44:11-13.
- NOVIKOV, V. A., A. GRECHUSHNIKOV, AND J. BARMENKOV. 1934. Accumulation of rubber in the roots of tau-saghyz as a result of its disappearance from the leaves. Compt. Rend. Acad. Sci. U.R.S.S. [N.S.] 1:205-207.
- PAUL, E. B., A. L. BLAKERS, AND R. W. WATSON. 1943. The rubber hydrocarbon of *Asclepias syriaca* L. Can. Jour. Research 21:219-223.
- POLHAMUS, L. G. 1933. Rubber content of various species of goldenrod. Jour. Agric. Research 47:149-152.
- , H. H. HILL, AND J. A. ELDER. 1934. Rubber content of two species of *Cryptostegia* and of an interspecific hybrid in Florida. U.S.D.A. Tech. Bull. No. 457.
- PROKOF'EV, A. A. 1939. Rubber formation in plants. Bull. Acad. Sci. U.R.S.S., Ser. Biol. No. 6, 908-923.
- . 1940. Biological role of rubber. Bull. Acad. Sci. U.R.S.S., Ser. Biol. No. 4, 589-607.
- . 1944. On the possibility of rubber formation by plants in heterotrophic nutrition with carbohydrates. Compt. Rend. Acad. Sci. U.R.S.S. 43:170-173.
- SAYRE, J. D. 1926. Physiology of the stomata of *Rumex patientia*. Ohio Jour. Sci. 26:233-267.
- SKAU, E. L., W. J. RUNCKEL, F. B. KREEGER, AND M. A. SULLIVAN. 1945. Physical chemical investigations of goldenrod rubber. III. The fractionation of goldenrod and other natural rubbers. Jour. Phys. Chem. 49:304-315.
- SPENCE, D. 1928. Recent scientific advances in connection with guayule. Rubber Age 23:133-134.
- . 1938. Preparation of latex from rubber-producing plants. U. S. Patent 2,119,030.
- , AND M. L. CALDWELL. 1933. Determination of rubber in rubber-bearing plants. Ind. Eng. Chem. Anal. Ed. 5:371-375.
- , AND W. J. MCCALLUM. 1935. The function of the rubber hydrocarbon in the living plant. Trans. Ins. Rubber Ind. 11:119-134.
- SYMONTOWNE, R. 1943. *Cryptostegia* research in Haiti. India Rubber World 108:148-150, 259-261.
- VISWANATH, B., et al. 1943. *Cryptostegia grandiflora* R. Br. A wartime source of vegetable rubber. Jour. Sci. Ind. Research (India) 1:335-383.
- WHITTENBERGER, R. T. 1944. Oil blue NA as a stain for rubber in sectioned or ground plant tissues. Sta. Tech. 19:93-98.
- , B. A. BRICE, AND M. J. COPLEY. 1945. Distribution of rubber in *Cryptostegia* as a factor in its recovery. India Rubber World 112:319-323.

STUDIES IN THE DEVELOPMENTAL ANATOMY OF PHLOX DRUMMONDII

HOOK. II. THE SEEDLING ¹

Helena A. Miller and Ralph H. Wetmore

IN PART I of this study (Miller and Wetmore, 1945) the successive changes in the development of the embryo of *Phlox Drummondii* Hook. were reported. From the fertilized egg, a radially symmetrical proembryo gradually is formed, possessing a well-defined epidermal layer, a cortex, and a procambial core. With the development of cotyledons, the radial symmetry gives way to bilateral symmetry in the heart-shaped embryo. At this

stage, the embryo possesses an epicotyledonary meristem, a procambial cylinder around a pith in the upper region, and the original procambial core, as well as a well-developed root meristem with a root-cap in the lower part. Progressive changes through a torpedo stage to the mature embryo showed the incidence of the first protoxylem and protophloem in the cotyledons.

Part II of this study is concerned with the subsequent changes in organization during seedling

¹ Received for publication July 2, 1945.

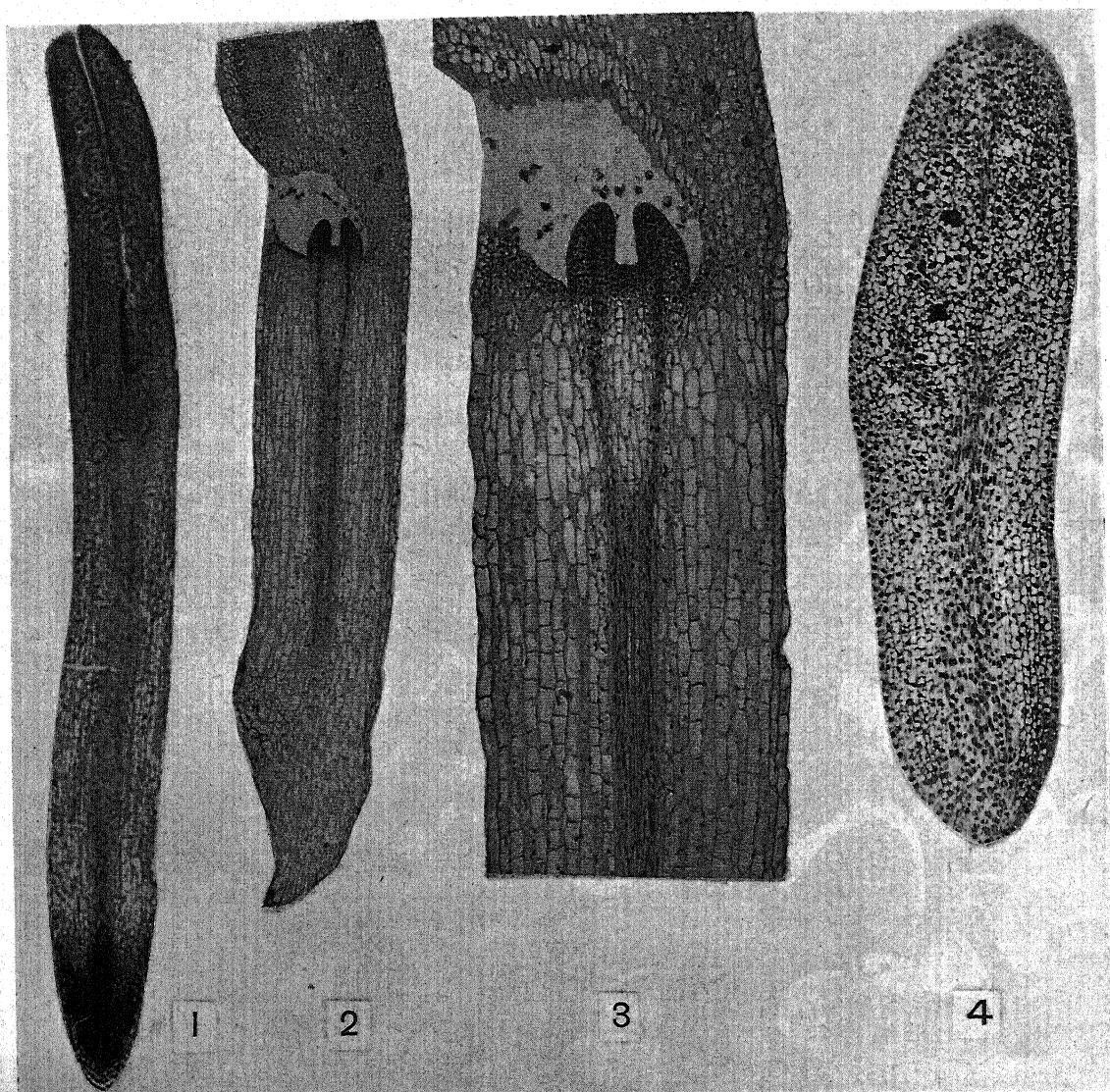


Fig. 1-4.—Fig. 1. Seedling just after germination. Note the elongate cells in hypocotyl. $\times 152$.—Fig. 2. Longitudinal section of a seedling about two days after germination. $\times 132$.—Fig. 3. Enlargement of figure 2. Note the procambial strands to the first pair of leaves. $\times 500$.—Fig. 4. Longitudinal section of an embryo before germination. Note the branching of the procambial strand in the cotyledon. $\times 360$.

development. The results provide the connecting links between the more generalized pattern of organization in the young embryo and the characteristic cylindrical pattern of the adult plant with its localized meristems in the apices of the shoots and roots (Part III).

The methods employed in this study were essentially those described for Part I. Progressively older stages of seedlings were utilized in an effort to complete the ontogenetic study.

OBSERVATIONS.—POST-GERMINATION STAGES.—The seeds were germinated on wet filter paper. In each, the hypocotyl elongates, pushing the root meristem through the micropyle. By placing marks of India ink at one mm. distances on such germinating seedlings, one can observe the pattern of growth. As the first marks became separated during the elongation of the plant, new marks of a different color were added to serve as further "land marks" for observation. From a typical record (fig. 5), it can be seen that in the early stages the root meristem (2) is moved away from the original mark (1) faster than the epicotyledonary meristem has advanced from that spot.

Microscopic examination shows that germination in *Phlox* results from an increase of cell length (greater first in the lower than in the upper hypocotyl) rather than from an increase in the number of cells (fig. 1). However, with the onset of ger-

mination, frequent cell divisions occur in the root meristem at the lower apex of the hypocotyl, thereby initiating the primary root.

As has been mentioned in the first part of this study, the recognition of protoxylem and protophloem mother cells in the procambium is possible while the embryo is in the torpedo stage. The protoxylem mother cells extend in continuous vertical files in the same radial plane throughout the whole length of the procambium from the tip of each cotyledon to the apex of the root. Their position in the upper cotyledon is mid-axial in the procambial strand; at lower levels of the cotyledon they are found more centrally in the strand. They then gradually come to occupy a peripheral position in the procambial cylinder of the hypocotyl and the procambial core of the root.

As the axis elongates during and after germination as a result of the activity of the root meristem, this solid core of procambium advances acropetally into the growing root, and behind it in turn, in *Phlox* at least, the protoxylem mother cells, which soon become protoxylem, also advance acropetally into this procambial core (fig. 6-9). The resulting xylem elements still maintain peripheral positions, one group on either side of the solid core directly below each cotyledon (fig. 12, X_1). The protophloem mother cells also extend the length of the plant (fig. 7-9), gradually becoming protophloem elements, but in a more oblique path than do those of the protoxylem.

In the hypocotyl, the protophloem mother cells are no longer in collateral association with the protoxylem mother cells; instead they gradually come to occupy positions on or near the periphery of the procambium on either side, midway between the protoxylem regions (fig. 12, P_1). Hence the fundamental pattern of the transition region between the collateral bundles of the cotyledon and the radial organization of the root is established in the torpedo stage before germination and even before any vascular elements are mature.

In the early post-germination stages it was noted from growth of marked specimens that elongation in the entire seedling took place more rapidly in the lower axis—the root—than in the upper axis—the hypocotyl. In later post-germination stages the rate of elongation of the root is reduced markedly and that of the hypocotyl is increased slightly (fig. 5). Accompanying this increase in length above the uppermost mark there is also an increase in rate of development in the epicotyl. On either side of the epicotyledonary meristem there appears a leaf primordium² in a position decussate with the cotyledons (fig. 2, 3).

Sections of the epicotyl show that elongate, dark-staining cells, traceable as procambial strands from the cylinder of the hypocotyl, are present as early

² As a matter of convenience, the first pair of leaves above the cotyledons is referred to as *the first pair*, the second pair above the cotyledons as *the second pair*, etc.

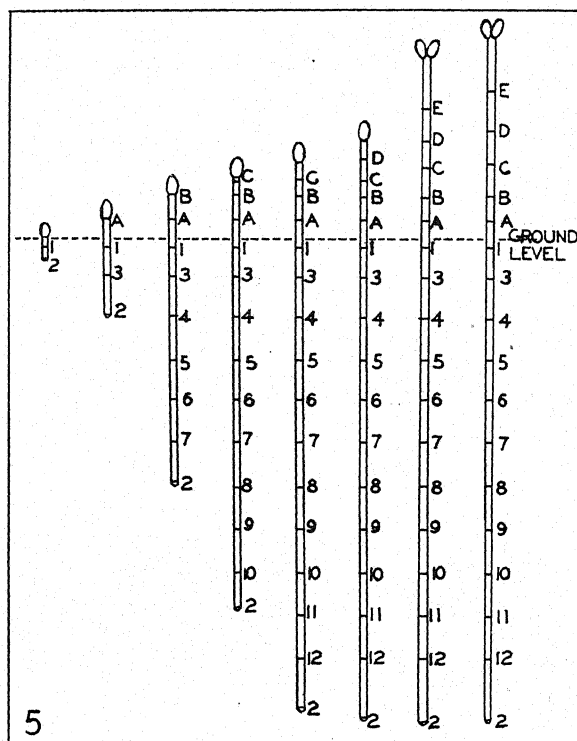


Fig. 5. Successive diagrams of seedling growth after germination. Marks were placed at millimeter distances. The junction of root and hypocotyl is just below (1) in each of the diagrams.

as the first indication of foliar elevation. These strands originate at opposite sides of the procambial cylinder in a plane at 90 degrees with the plane of the cotyledons. In a great many of the seedlings studied, discontinuity in the procambium leading to the leaves (fig. 2, 3) was never noted. As each leaf elongates by the activity of the meristem at its apex, the procambium also becomes extended acropetally. During this elongation of the foliar primordia with their accompanying procambial strands, the abaxial parenchyma of the leaf becomes slowly and progressively more vacuolated. Adaxial vacuolation is slower in its appearance.

Subsequently there follows an acropetal maturation of protophloem from the mature protophloem in the cylinder below (fig. 10). The position of this protophloem changes gradually as one follows it from the solid core upward to the cylinder. In the solid core it occupies the outer portions of an imaginary arc which is broader than, and centrad to,

that arc in which the cotyledonary protophloem occurs (fig. 12 j, P 2). In the cylinder, this inner arc becomes narrowed and less central at a level where the outer phloem arc splits into two, one half passing to each cotyledon. Still higher, the inner arc constitutes the phloem part of the strand to the first leaf, and occupies the abaxial part of the entire bundle (fig. 12 g, h).

The maturation of xylem elements in these leaves, contrary to the condition in the cotyledons, occurs after the maturation of the first protophloem elements. The locus of the initial maturation of protoxylem is on the mid-adaxial side of the procambial strand of the leaf at a level just above the leaf base. The wave of maturation progresses from this locus acropetally to the leaf tip and basipetally into the region where mature xylem elements subjacent to the cotyledons are already present in the hypocotyledonary cylinder (fig. 11). The cylinder in the hypocotyl axis, formerly all of procambium,

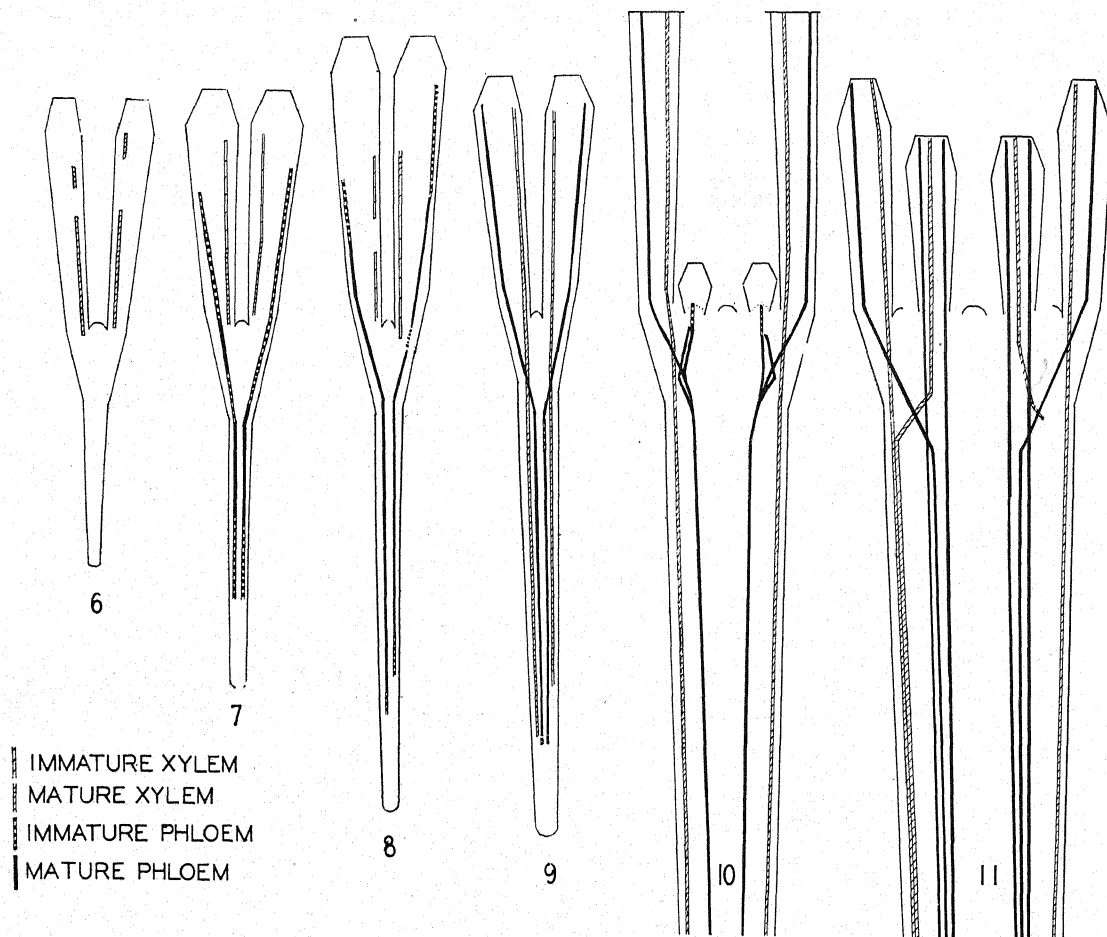


Fig. 6-11. Diagrams of embryo and seedlings constructed from cross sections to illustrate vascular maturation.—Fig. 6. Diagram of embryo before germination.—Fig. 7-9. Diagrams of successively older seedlings.—Fig. 10-11. Diagrams of successively older seedlings with one pair of epicotyledonary leaves. In figures 10 and 11 the first pair of leaves are placed in the same plane with the cotyledons to make the diagram more easily interpreted. The first pair of leaves is actually decussate with the cotyledons on the plant itself. The limitations of this type of diagram do not facilitate illustration of the xylem and phloem as occupying radial planes in the root which are different from those occupied in the cotyledon and leaves. See text and figure 12.

is now composed of one trace to each cotyledon and another to each of the first pair of leaves, the traces of this pair of leaves being decussate with those of the cotyledons (fig. 12 i). Lower in the seedling the traces become a part of the solid core (fig. 12 k). Between these two levels the xylem part of each leaf trace for the first pair of leaves gradually appears separated into two portions, each continuous with those xylem elements now being differentiated on the flanks of the adjacent cotyledonary traces (fig. 12, X₂ i-k). Thus, the xylem portion of each first leaf seems to split in the transition zone, at about the top of the solid core; actually it has matured basipetally in the procambium in these regions. Lower in the solid core the xylem elements from each of the first leaves occupy the two areas between the phloem and the protoxylem points on one side of the metaxylem plate; those from the other lie in similar positions on the other side of the plate. The remaining procambium will in time become incorporated into the vascular aggregates of future leaves and buds.

The second pair of leaves is decussate with the first pair, and hence lies directly above the cotyledons. The procambial strands to this second pair of leaves can be traced from the procambium below. They are acropetal extensions from those four procambial areas which in the core lie between the protoxylems and protophloems of the first pair of leaves, and which in the cylinder flank the cotyledonary traces on either side. The maturation of protoxylem and protophloem in the procambial system of the second pair of leaves progresses just as in the case of the first pair of leaves; that is, the protophloem advances acropetally; the protoxylem is initiated at, or near, the leaf base and develops both acropetally and basipetally.

In the cylinder, at levels below the emergence of the second pair of leaves, the procambium supplying each of this pair lies in regions adjacent to the vascular tissue of the cotyledons. In the region above the emergence of the cotyledons, the procambium from either flank of the cotyledonary strand gradually comes to occupy adjacent positions in the single strand supplying each leaf. Hence there is formed a so-called "leaf gap." The somewhat retarded adaxial vacuolation of the cotyledons becomes evident only when the second pair of leaf primordia is forming. A cotyledonary gap is completed when the adaxial vacuolation of the parenchyma in the cotyledons has become continuous with the pith across the cortex, along the upper side of the cotyledonary trace.

As the third pair of leaf primordia is formed, the vacuolation on the adaxial side of the first pair becomes complete and a "gap" is formed for each of that pair of leaves. As the fourth pair of leaf primordia appears, the leaf gaps are formed for the second pair of leaves; and the elongation of the epicotyl between the cotyledons and the first pair of leaves causes the formation of a recognizable internode.

DISCUSSION.—The seedling stage of angiosperms has received a great deal of attention in botanical literature because of the many significant changes

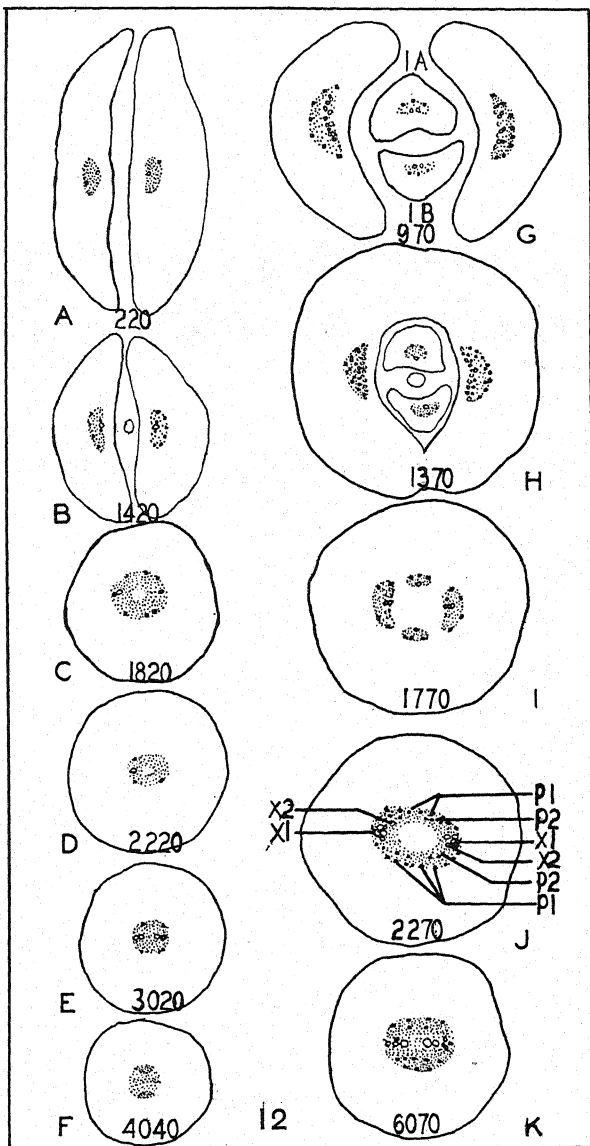


Fig. 12. Camera lucida drawings of transverse sections at certain levels of seedlings, showing positions of xylem and phloem. $\times 143$. Seedling a-f—with no leaves present; seedling g-k—with one pair of epicotyledonary leaves present. X = xylem; P = phloem; PI = xylary plate; X₁ and P₁ = xylem and phloem respectively from first pair of leaves.

which occur in the plant during this period. Among the important aspects which are associated with this stage of development in *Phlox* must be listed: the germination of the seed, the establishment of a root, the elongation of the hypocotyl, the establishment of an epicotyl with its leaves and characteristic leaf arrangement, and the development of the vascular

pattern in the seedling. Of special interest to botanists has been also the interpretation of the vascular pattern in the transition region. Of these topics, only two need added comment beyond the descriptive facts of the text: the development of the vascular pattern of the epicotyl and the interpretation of the transition zone.

It has been pointed out in Part I of this series of papers that the procambium becomes differentiated in the young axillary symmetrical embryo and develops acropetally with the formation of the cotyledons and basipetally with the formation of the root. With the formation of the epicotyl, as described in this paper, the procambium develops acropetally into the leaves from the already continuous procambium below. This is in accordance with the report of acropetal differentiation reported in *Alstroemeria* leaves by Priestley, Scott and Gillett (1935); in *Sequoia* shoots by Crafts (1943); in *Acacia* phyllodes and in the cauline bundles of *Trichocereus spachianus* and *Opuntia cylindrica* by Boke (1940, 1941) as well as in *Vinca rosea* (Cross and Johnson, 1941) and *Cunninghamia lanceolata* (Cross, 1942).

The problem of the transition region is complicated in part by the existence of two concepts as to its meaning. The term has been used in a general way to refer to that region of a seedling in which the top of the primary root is confluent with the bottom of the shoot axis. It has also been used to refer to a specific part of this region in which there is a transition from the radial arrangement of phloem and exarch xylem in the root to the (usually) eustelic cylinder of collateral bundles in the shoot.

It is possible to consider the transition region according to the first concept without considering any vascular pattern. The position of this region in *Phlox* is predicated in the young embryo. It can be demarcated in such an embryo by the extent of the pith between the solid core and the cotyledonary node. The ultimate length of the region is dependent upon the amount of growth occurring in the upper hypocotyl between the torpedo stage of the embryo and that stage of seedling development in which there is no further elongation in the hypocotyl. The transition region in *Phlox* is similar to that in *Daucus Carota* (Esau, 1940; Havis, 1939) and in *Tragopogon porrifolius* (Havis, 1935). In those embryos in which no solid vascular core is reported, e.g., *Juglans* (Nast, 1941), the transition region is not completed until after the solid core of the root has been produced by the activity of the root meristem.

Most of the literature deals with the more specific concept of the transition zone. Lehmberg (1923-24) attempted to explain the change of the xylary pattern in the transition zone of *Helianthus* seedlings as resulting from two centers of differentiation of xylem, one in the upper root, the other in the cotyledons. From the former, maturation progresses in both directions, later-formed elements developing in a centripetal direction. From the latter, it also progresses acropetally and basipetally, but later-formed xylem elements are developed in a centrifu-

gal direction. Where the two meet and become a functional water-conducting system, the transition is completed.

Chauveaud (1911) points out that the early differentiation of xylem and phloem in a great many plants seems to be initiated in the lower hypocotyl or in the root of the seedling, in alternating radii—the *alternate arrangement*. At higher levels in the early stages he reports additions on the flanks of the xylem strand—the *intermediate arrangement*. In the region of the cotyledons, or just below them in the upper hypocotyl, he reports only a slender extension of the xylary bundle from the root, which is flanked laterally, or laterally and abaxially by two half-strands of phloem—the *superposed arrangement*. Branches from this system in the cotyledons also have this superposed arrangement. The phloem half-strands become connected laterally in the upper cotyledons, and later also in the lower cotyledons by the obliteration of the slender xylary extension between them. Thus the superposed condition exists in the main vein of the cotyledons.

Chauveaud describes the protoxylem as being continuous as a small strand in a young seedling. It is increased in size by the addition of new elements centripetally below and laterally and centrifugally above. Thus the latest-formed xylem elements do not lie in a continuous vertical file as did the earlier-formed protoxylem.

Chauveaud accounts for some of the variation found in the numerous genera studied by the obliteration of early-formed elements of xylem at different stages of growth. He suggests that the pattern of development in ontogeny is not unlike that in the evolutionary sequence.

In this study of *Phlox* it was also found that a continuous file of xylary elements exists throughout the seedling and the addition of new elements occurs just as Chauveaud describes. However, the first protoxylem elements to be matured lie in the base of the cotyledons in this material, rather than in the lower hypocotyl or root. The establishment of a continuous file of mature protoxylem elements occurs by progressive and rapid basipetal maturation, rather than by the basifugal maturation reported by Chauveaud. The obliteration of elements was not observed in the stages of *Phlox* studied.

Those who have interpreted the transition region in terms of the xylary pattern have considered the pattern as it is represented by mature elements. In none of the literature which has come to the authors' attention has there been any mention of a stage of development between the procambial cell and the immature protoxylem, nor to any intermediate stage between the procambial cell and the immature proto-phloem element. In this study it was noted that the future xylem regions of the bundles could be detected, because of an artifact of fixation, in embryos much smaller and younger (the torpedo stage), than in those in which the first mature protoxylem cells were found (the late embryo). Future phloem regions could also be detected in a similar manner.

TABLE 1. Vascular counts at certain levels of the seedlings illustrated in figure 12.

Seedling 12 A-F					Seedling 12 G-K								
Microns from top of plant	Left cotyledon		Right cotyledon		Microns from top of plant	Left cotyledon		Leaf 1a		Leaf 1b		Right cotyledon	
	xylem	phloem	xylem	phloem		xylem	phloem	X.	P.	X.	P.	xylem	phloem
220	(1) ^a	1	1	2	370	0	0	0	1
620	1	2	2	3	770	17	7	3	1	2	2
1020	2	4	4	4	970	17	10	2	1	2	2	12	7
1420st. ^a	3	4	3	6	1170	17	11	1	4	1	4	17	11
1820	2	3	1	4	1370st. ^a	24	17	1	4	1	4	19	16
2220	1	2	1	6	1570	19	10	1	4	1	4	16	12
2620	2	3	1(1) ^a	2	1770	18	9	1	3	1	3	10	8
2820	4	3	3	3	2270	20	10	1	4	1	5	15	9
3020	3	2	3	2	2470	15	10	0	5	0	6	14	9
3420	3	2	1	2	2970t.l. ^a	16	10	0	6	0	5	10	8
<div style="text-align: center;">↔</div>													
3820	0	2	0	1		↔				↔			
4220	0	0	0	0	3970	9	↔		9	..	8	10	..
....	4870	9	9	..	7	11	..
....	5670	10	7	..	6	7	..
....	6070pl. ^a	5	6	..	10	7	..
....	6870	9	7	..	5	9	..
....	7870	7	3	..	3	8	..
....	8870	3	2	..	5	4	..
....	9970	2	2	..	2	2	..
....	10000	0	0	..	0	0	..

^a st. = stem tip; t.l. = traces "lost"; pl. = xylary plate evident; (1) = element immature.

Thus in *Phlox* at least, it appears that the alternate arrangement of xylem and phloem in the lower hypocotyl, the intermediate arrangement in the upper hypocotyl, and the superposed condition in the cotyledons can be detected before any elements are matured. It follows, then, that the order of maturation of protoxylem and protophloem does not necessarily duplicate that order of the earlier appearance of their precursors in the blocking out of the vascular pattern. This situation can be compared with that in roots, where the xylem pattern is early blocked out in a centrifugal order, and later followed by a progressively centripetal sequence of maturing xylem cells (Williams, 1940; Esau, 1940). Another similar situation has been reported for the stems of *Lycopodium* (Wetmore, 1943).

The relative numbers of mature xylem and phloem elements at a given level in a young leaf as compared to those at other levels in that leaf have been used to indicate not only the maximum maturation, but also the place of origin of the first mature element in that organ (Esau, 1938, 1943). In the vegetative apices of *Phlox*, it has been found that the criterion of greatest numbers of mature elements does point out both the center of maximum maturation and the locus of maturation of the first element. In at least the hypocotyl and cotyledons of a young seedling, however, the place of greatest numbers of mature xylem elements (fig. 12 and table 1) was not the locus of the maturation of the first elements (Miller and Wetmore, 1945).

It would seem plausible to the authors, in the light of the observations here reported, that attention

should be directed to premature stages of development of vascular elements in any approach to the problems related to patterns in development.

SUMMARY

The seedling develops first by an increase in growth of the lower part of the embryo of the mature seed, causing the germination of the seed. After germination, the seedling grows by activity both at the root apical meristem, extending the root, and at the shoot apical meristem, producing successive pairs of decussate leaf primordia.

The high transition region in *Phlox* is correlated with the existence of a solid core of procambium in the early stages of the embryo. A pith-containing cylinder appears in that region which is formed at the tip of the embryo after the torpedo stage. The extent of the transition region depends upon the amount of growth in this pith-containing region of the embryo and seedling.

The pattern of the transition region in the seedling is foreshadowed in the embryo by regions of potential xylem and potential phloem blocked out in the procambial system before any elements are mature in either of these regions. This blocking out does not seem to occur until some pith has been formed by the rib meristem at the base of the epicotyledonary meristem (Part I).

A continuous file of mature protoxylem cells is established within the procambial system, progressing in both directions from the point of first maturation in the base of the cotyledon. Additional matured xylary elements are formed in a centripetal

direction in the lower hypocotyl and root, but in a centrifugal direction in the cotyledons.

A continuous file of mature protophloem cells is established within the procambial system progressing in both directions from the point of its first maturation in the base of the hypocotyl. Successive phloem elements mature in a centripetal direction throughout the length of the plant.

In the seedling the locus of greatest numbers of matured elements is not identical with the point of first maturation. This is in contrast with the condition found in vegetative and floral apices (Part III).

BIOLOGICAL LABORATORIES,
HARVARD UNIVERSITY,
CAMBRIDGE, MASSACHUSETTS

LITERATURE CITED

- BOKE, N. H. 1940. Histogenesis and morphology of the phyllode in certain species of *Acacia*. Amer. Jour. Bot. 27: 73-90.
- . 1941. Zonation in the shoot apices of *Trichocereus spachianus* and *Opuntia cylindrica*. Amer. Jour. Bot. 28: 656-664.
- CHAUVEAUD, G. 1911. L'appareil conducteur des plantes vasculaires et les phases principales de son évolution. Ann. Sci. Nat. IX, Bot. 13: 113-438.
- CRAFTS, A. S. 1943. Vascular differentiation in the shoot apex of *Sequoia sempervirens*. Amer. Jour. Bot. 30: 110-121.
- CROSS, G. L. 1942. Structure of the apical meristem and development of the foliage leaves of *Cunninghamia lanceolata*. Amer. Jour. Bot. 29: 288-301.
- , AND T. J. JOHNSON. 1941. Structural features of the shoot apices of diploid and colchicine-induced tetraploid strains of *Vinca rosea* L. Bull. Torrey Bot. Club 68: 618-635.
- ESAU, K. 1938. Ontogeny and structure of the phloem of tobacco. Hilgardia 11: 343-424.
- . 1940. Developmental anatomy of the fleshy organ of *Daucus carota*. Hilgardia 13: 175-226.
- . 1943. Vascular differentiation in the vegetative shoot of *Linum*. II. The first phloem and xylem. Amer. Jour. Bot. 30: 248-255.
- HAVIS, L. 1935. The anatomy and histology of the transition region of *Tragopogon porrifolius*. Jour. Agric. Res. 51: 643-654.
- . 1939. Anatomy of the hypocotyl and roots of *Daucus carota*. Jour. Agric. Res. 58: 557-564.
- LEHMBERG, K. 1923-24. Zur Kenntnis des Baues und der Entwicklung der wasserleitenden Bahnen bei der Sonnenblume (*Helianthus annuus*). Bot. Centrbl. Beihefte 40: 183-236.
- MILLER, H. A., AND R. H. WETMORE. 1945. Studies in the developmental anatomy of *Phlox Drummondii* Hook. I. The embryo. Amer. Jour. Bot. 32: 588-599.
- NAST, C. G. 1941. The embryogeny and seedling morphology of *Juglans regia* L. Lilloa 6: 163-205.
- PRIESTLEY, J. H., L. I. SCOTT, AND E. C. GILLET. 1935. The development of the shoot of *Alstroemeria* and the unit of shoot growth in monocotyledons. Ann. Bot. 49: 161-179.
- WETMORE, R. H. 1943. Leaf-stem relationships in the vascular plants. Torreya 43: 16-28.
- WILLIAMS, B. C. 1940. Differentiation of vascular tissue in root tips. Abstract. Amer. Jour. Bot. 27: 10s.

THE WATER FACTOR IN TRANSPLANTING GUAYULE¹

Louis C. Erickson

IT is well known that water relations are important from the standpoint of recovery of transplants, and several practices are commonly employed to keep water loss at or below water uptake. These include (1) hardening of the plants, (2) retention of as many roots as possible, (3) removal of part of the top, (4) avoidance of drying between digging and planting, (5) planting in moist soil and watering immediately, (6) protection from wind and sunshine, and (7) planting during cool, humid weather. Weaver and Clements (1929) and Bailey (1939) state that the repeated transplanting of a plant makes the process more successful. This practice results in a compacting of the root system so that larger proportions are transferred. Weaver and Clements (1929) recommend that tops be pruned so as to be in balance with the roots retained on the transplants. Kraus (1942) found that while it was unnecessary to top or partially defoliate certain vegetables at the time of transplanting to increase survival, failure to water transplants until the ninth day resulted in about fifty per cent stands.

In the machine-planting of guayule on a com-

mercial scale it is the practice, for obvious practical reasons, to use only about six inches of a tap root system that may be from four to ten feet long². The removal of 85 to 95 per cent of the length and probably even more of the absorbing area is severe treatment. To compensate for this it has been found beneficial to prune the top severely. Lloyd (1911) stated that the removal of all of the top of a guayule transplant increased chances of survival, and recently Smith (1944) obtained quick recovery and high survival with slightly less severe topping.

The object of this investigation was to determine whether or not the value of topping in hastening the establishment of transplants of guayule was associated with its effect on the water relations of the plant.

METHODS AND MATERIALS.—This study consisted mainly in the determination of changes in fresh weight or water content of the plants. Plants were transplanted to soil (field) or water (in greenhouse and outdoors). In the case of the plants in soil, moisture determinations of the plants were made

² Unpublished data of C. H. Muller, associate botanist, Special Guayule Research Project.

¹ Received for publication July 10, 1945.

by harvesting and drying the plants at 100°C. For plants in water, changes in fresh weights were determined and in some experiments the plants were also dried at 100°C. to determine percentages of moisture. All weighings were made on a torsion balance and weights were recorded to the nearest .01 gm.³

In the water culture experiments tap water was used without addition of minerals. Containers were of three sizes: six-inch test tubes (80 ml. cap.), three-gallon glazed jars, and one-by-two-by-four foot wooden tanks coated with asphalt paint. The plants were held singly in paraffined corks of two inches in diameter and one-half inch thickness. Some experiments involved the control of relative humidity, and also girdling of stems and roots. Girdling was done by cutting the bark in two places with a knife and removing the intervening segment.

Nursery plants fifteen to eighteen months old in a drought-dormant condition were used for most of the experiments. The plants were grown in the U. S. Forest Service Nursery at Salinas, California. Seeds were sown May 8, 1943, and cultured by the standard procedure at the time (i.e., abundant watering). After September of 1943 the plants were allowed to harden in drying soil and decreasing temperatures. Further growth in height did not occur until April and the first part of May, 1944. Before more than about one-half to one inch of new stem growth had taken place the seedlings became drought-dormant. To prevent shriveling during June and July the plants were given one-half inch of water in each of two applications by the overhead irrigation system. Transplantings of these plants to the field during the summer gave 95 to 100 per cent sprouting of buds within three weeks after planting. The plants used were cut back to have six-inch tap roots and two-inch leafless tops. The size range prior to topping was from three-sixteenths to three-eighths of an inch in crown diameter and from five to eight inches in height of stem.

In the following experiments, unless otherwise stated, plants of the above type were used and tap roots were cut to a six-inch length, and all lateral roots removed. Tops were treated as will be described for each experiment (fig. 5).

Air temperature and relative humidity records were obtained for the experimental periods in greenhouse and field. In the greenhouse the lowest maximum was 76°F. and the highest maximum 92°F.; the lowest minimum was 57°F. and the highest minimum 68°F. In the field the same determinations were 63, 92, 43, and 57°F., respectively. Relative humidities in greenhouse and field ranged from the forties or fifties to 100 per cent practically every day and dews were not uncommon in the field.

EXPERIMENTAL.—Effects of topping on changes in fresh weight.—In preliminary experiments it was noted that untopped transplants tended to shrivel even though their roots were in water. To measure

such changes the following experiment was initiated. Twenty-five nursery plants were dug on

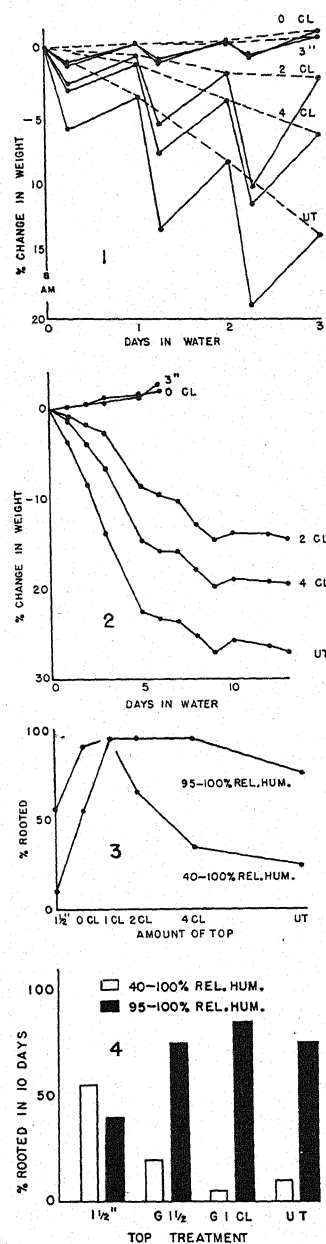


Fig. 1-4.—Fig. 1-2. Mean changes in weight of five guayule transplants in each of five topping levels after being placed in tap water. CL and UT denote clusters of leaves remaining and untopped, respectively. In figure 1, morning and afternoon determinations are shown, while in figure 2 only those for mornings are given.—Fig. 3. Percentages of plants rooted in greenhouse chambers after nine days in water when several degrees of topping and two conditions of humidity were used. Each value based on twenty plants.—Fig. 4. Relative effects of girdling and humidity. Top treatments were: topped 1½ inches above crown (1½"), girdled 1½ inches above crown (G 1½"), girdled above a branch with one cluster of leaves (G 1 CL), and untopped (U T). Each value based on twenty plants.

³ Smallest plant (topped 1½") was 1.83 gms.; mean of all, 5.98 gms.

August 2 and randomized into five groups for the following topping treatments: tops cut to three inches above crowns (no leaves remained); not topped, but all clusters of leaves removed; all but two clusters of leaves removed; all but four clusters of leaves removed; and tops unchanged (untopped). The roots were immersed in tap water contained in three-gallon jars. Weighings of the individual plants were made morning and afternoon for three days and subsequently in the morning until the thirteenth day. Paper towels were used to dry off the excess water from the tap roots prior to weighing. The drying was consistent to within .02 gm. (<1 per cent of wt.) of the mean determined by repeated wetting, drying, and weighing of individual plants.

The results of the experiment show that plants receiving each of the topping treatments lost weight during the daytime and gained weight at night (fig. 1). The amount lost during the daytime was closely related to the number of clusters of leaves present, being most for the untopped plants. Nightly gains in weight were not equal to the losses, however, and so definite trends were found. In the case of plants topped to three inches and also those in which no clusters of leaves remained, the nightly gains exceeded daytime losses, resulting in a net increase in weight. On the other hand, the plants with clusters of leaves present failed to regain the daily losses and consequently the trend was in the direction of decreasing weight. Analysis of variance (Snedecor, 1940) for weights in the third morning showed highly significant differences ($F = 43.48$; 1 per cent probability = 4.43). New roots began to appear during the third day and by the fifth day all ten plants without leaves had new root tips emerging. None of the other plants rooted⁴ within the time limits of the experiment. The initial rate of decrease in maximum weight from day to day was

⁴ *I.e.*, formed new roots. Here and elsewhere in the paper *rooting* and *rooted* refer to the regeneration of root tips on the portions of root systems transplanted, which without exception at the beginnings of the experiments were devoid of any outwardly visible apices.

not maintained by the leafy plants, so that the maximum weights tended to become constant after a few days (fig. 2). The important points of the experiment were the rapidity with which changes in fresh weights occurred and the association of these changes with the subsequent rooting responses of the plants. Changes in weights were largely due to net water loss and gain, as will be shown in the next experiment.

Water uptake and water loss.—Eighty plants were dug on the afternoon of August 14 and taken to the greenhouse. They were divided about equally between two three-gallon jars containing enough water to cover the roots. After remaining overnight in the jars, they were placed in eighty test tubes containing water. The tubes, with spacing coinciding with that of the holes in the lids used for the three-gallon jars of the previous experiment, were held upright in vials sunk into sand. Thus, the plants were held by corks in a manner common to other experiments but in the place of a large volume of water under the lids, there were test tubes of water into which the roots extended. The purpose of the lids and corks was mainly to remove the direct solar radiation from the roots.

The treatment of tops was in terms of the number of clusters of leaves remaining: 0, 1, 2, and 4. The treatment of roots was in terms of the number of two-inch lateral roots on the six-inch tap root at the beginning of the experiment: 0, 1, 2, and 4. Thus there were sixteen treatments of five plants each. Five test tubes without plants were included to obtain an approximation of the evaporation of water. The arrangement of plants was in five randomized blocks.

The lateral roots that were retained were about one millimeter in diameter and care was taken to select ones in which transverse fissures were absent. Preparation of the plants required most of the day, so that weighings were not started until the next morning (August 16). The presence of the lateral roots made it necessary to shake the excess water off rather than to dry the roots with towels before weighing (as in the previous experiment). Varia-

TABLE 1. *Water uptake, water loss, and rooting of guayule transplants during a five-day period in water culture when numbers of clusters of leaves and two-inch lateral roots on the transplants were varied (each value based on twenty plants).*

	I. Grouped by clusters of leaves					II. Grouped by lateral roots				
	Number of leaf clusters				Amt. for signif. at 1% ^a	Number of lateral roots				Amt. for signif. at 5%
	0	1	2	4		0	1	2	4	
Mean water uptake, gms.....	2.32	4.45	5.20	5.92	...	4.17	4.63	4.45	4.64	...
Mean water loss, gms.....	2.30	4.69	5.68	6.64	...	4.44	4.96	4.81	5.09	...
Change in fresh weight per plant, gms.	+ .02	— .24	— .48	— .72	...	— .27	— .33	— .36	— .45	...
Mean % change in fresh weight..	+ .51	— 3.47	— 6.50	— 9.27	2.31	— 3.76	— 4.38	— 4.50	— 6.10	1.74 ^b
% rooted	100	30	10	5	...	30	25	45	45	...

^a Hayes and Immer (1942), p. 311.

^b Observed $F = 2.11$; amount expected at 5% point = 2.75. By the analysis of variance method the means did not differ significantly.

TABLE 2. Mean per cent moisture content of guayule transplants after a total of six and one-half days in water culture.

	I. Grouped by clusters of leaves						II. Grouped by lateral roots					
	Number of leaf clusters				Amt. req. for signif.		Number of lateral roots				Amt. req. for signif.	
	0	1	2	4	5%	1%	0	1	2	4	5%	1%
Leaves	60.4	56.6	54.5	3.11	4.16	56.1	56.9	58.9	56.4	3.11	4.16
Stems	53.2	50.3	48.3	45.5	1.53	2.03	49.2	48.8	49.8	49.4	1.53	2.03
Roots	58.5	55.8	53.9	51.7	1.32	1.76	55.1	54.6	55.4	54.9	1.32	1.76

tions were found to be not more than .04 gm. (<2 per cent of wt.) from the mean of several successive determinations.

The first weighing was taken as the weight upon which to base changes during the subsequent five days. The plant weight was determined first, followed by a determination of the weight of plant, plus water and container. At the next weighing the total weight was determined first, followed by a determination of the weight of the plant alone. After replenishing the water the total weight was again recorded. The mean water loss from the five test tubes without plants was used as a correction factor for the test tubes with plants. Reduction of the surface area of the water in the test tubes by the plants was perhaps overcompensated for by the capillary upcreep of water on the stems. However, the plants were treated alike so that relative differences were considered reliable even if absolute determinations were in slight error.

Reweighings were made at 24, 48, and 120 hours after the initial determination. When the plants were grouped by clusters of leaves remaining on the plant, trends in fresh weights were similar to those of the first experiment, but when they were grouped by the number of lateral roots present at the beginning of the experiment, there was no significant difference (table 1). In the case of the plants with no clusters of leaves, the water uptake exceeded water loss, while in the case of the leafy plants, water loss exceeded water uptake. The variation in number of lateral roots as used in this experiment reflected no significant change in water uptake. The highly significant differences found in water uptake were due only to the topping treatments. Interaction between leaves and roots was not statistically significant ($F = .82$). As the rate of water loss increased with increased amounts of leaf tissue present, the rate of water uptake also increased but not as rapidly.

An indication of rooting was first found after about four days in water. At harvest (six and one-half days in water) all plants without leaves were rooted, but as in the previous experiment, the leafy plants lost a great deal of fresh weight and failed to root in appreciable numbers (table 1). Moisture content determinations were made on leaves, stems, and roots (laterals removed) of all the plants (individually). The moisture contents showed that the losses recorded in fresh weights were the results of

losses from tap roots as well as stems and leaves (table 2).

Regions of water loss in roots.—To determine whether the decrease in moisture content of roots was due to desiccation of bark cells (all cells exterior to the cambium) or simply to an emptying of water-conducting elements of the wood the following experiment was undertaken on October 21.

Thirty plants were used, ten to obtain initial moisture of roots and twenty to determine moisture content after five days in water. Those which were to be placed in water were divided into two groups: untopped and topped to three inches. Moisture contents of roots were determined for bark and wood separately, two plants in each of five replications.

The decrease in moisture on the part of roots of untopped plants was due to loss in both bark and wood (table 3). When it is taken into account that

TABLE 3. Changes in moisture content in bark and wood of roots of guayule transplants in water culture.

	After 5 days in water		
	Initial	Topped to 3"	Untopped
	%	%	%
Bark	63.9 \pm 1.1 ^a	66.1 \pm .5	60.1 \pm .3
Wood	37.2 \pm .5	36.8 \pm .3	33.9 \pm .5
Weighted mean...	53.2 \pm .6	55.6 \pm .4	50.1 \pm .4

^a Standard error.

percentages of moisture are based on fresh weight, then the change from 63.9 per cent to 60.1 per cent in the bark involved about twice as much water per unit dry weight as the change from 37.2 per cent to 33.9 per cent in the wood and since dry weights for bark and wood of the roots were nearly equal, about two-thirds of the loss in moisture content was due to loss of water from the bark.

Lateral roots and root regeneration.—Transplants vary considerably with regard to the number of lateral roots retained. Some measure of their importance to the transplants may be obtained from the following experiment. One hundred nursery plants with many lateral roots were obtained August 11 and placed with their roots in three-gallon jars of aerated tap water, four plants per jar. All plants were left untopped but their roots differed in respect to the number of lateral roots remaining: 0, 2, 4, 8, and x. In plants with 2, 4, and 8 laterals

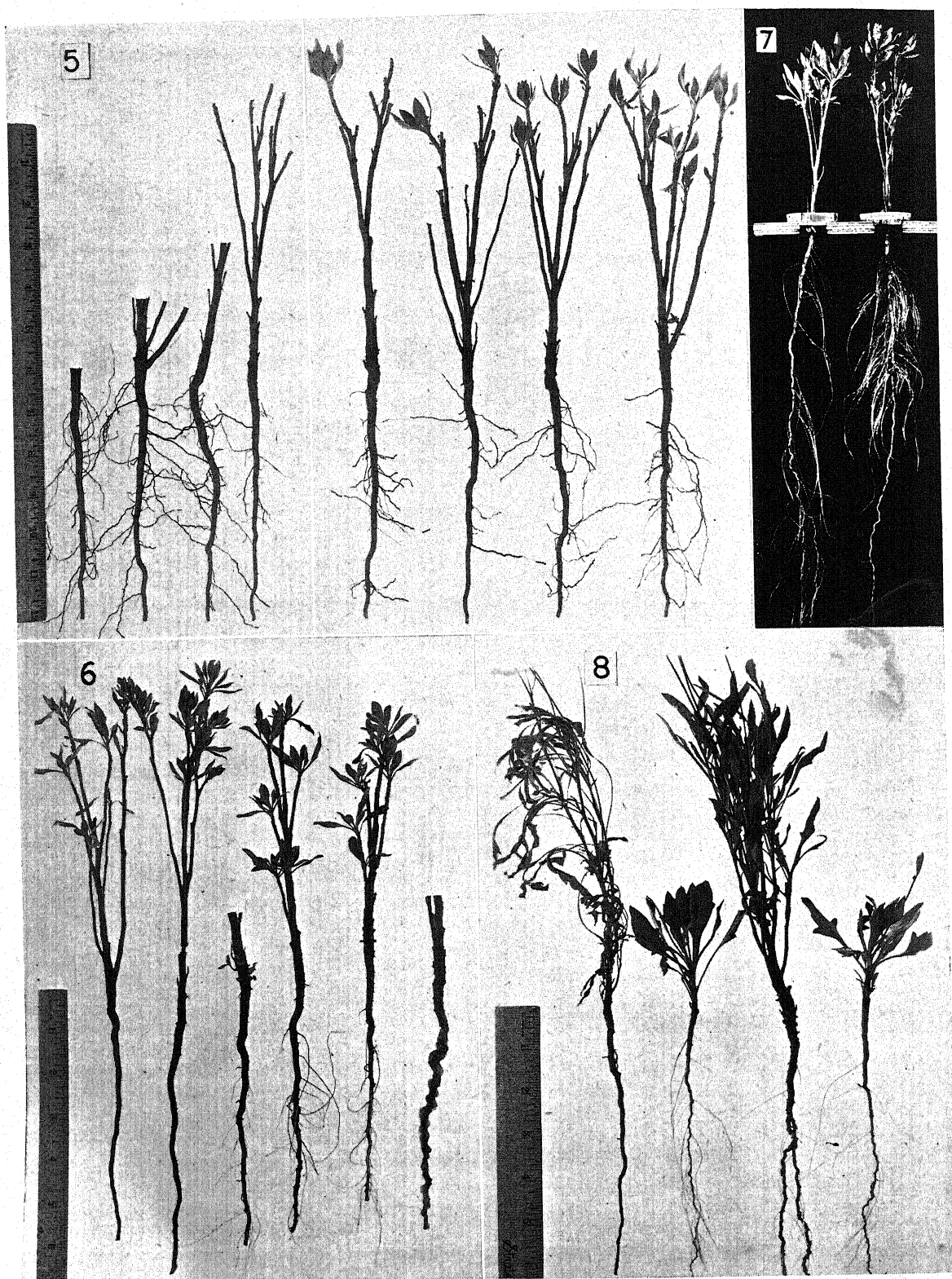


Fig. 5-8.—Fig. 5. Types of topping used in experiments. Left to right: (C) crown; ($1\frac{1}{2}$ " $1\frac{1}{2}$ inch of stem; (3" 3 inches of stem; (OCL) all clusters of leaves removed; (1 CL, 2 CL, 4 CL) all but one, two or four clusters of leaves removed; (UT) untopped or no clusters of leaves removed. Note variable condition of lateral roots in representative nursery stock.—Fig. 6. Effect of girdling and relative humidity. The three plants on the left were kept at

these were cut to two inches in length. In plants with x laterals the maximum amount of root system was retained both as to length and numbers of laterals. The rooting was at a maximum on the eleventh day with 5, 15, 25, 60 and 95 per cent of the plants rooted, respectively, for the above groups. As in the previous experiment in which the number of lateral roots was varied, the amount of rooting showed little increase when four or fewer lateral roots were retained, but when eight or more were retained, the rooting was considerably increased.

Five plants for each treatment were weighed to determine changes in fresh weights during the first ten days of the experiment. It was found that plants subjected to all treatments lost weight. Thus, even the presence of many laterals did not increase uptake of water to the point where it was as rapid as water loss.

The presence of laterals was important because the first new roots in most cases appeared on them. It is not known at what rate the lateral roots desiccated, if at all. The rapid rooting, when it did occur, suggests that new roots developed before water became a limiting factor.

On the fifth day it was found that the plants with many lateral roots deviated considerably from those subjected to other treatments in regard to loss in weight. On the tenth day the deviation was even greater, indicating that the weight of new roots and increased water uptake were appreciable.

Relative humidity.—To check further on the factor of water loss as influencing rooting, an experiment was set up in which plants were put into glass chambers in the greenhouse. One chamber was incompletely closed so as to have about the same relative humidity (40 to 100 per cent) and temperature as the greenhouse, while the other chamber was supplied with fog from a "mistifier" to keep the relative humidity continuously high (95 to 100 per cent). Two hundred and forty plants were used, one-half under each condition of humidity. Six types of topping with twenty plants in each group were used: untopped; 4, 2, 1, and 0 clusters of leaves remaining; and $1\frac{1}{2}$ inches of stem. The plants were randomized but closely spaced at twenty-four plants for each three-gallon jar. Lids with many small holes evenly spaced were used to hold the plants upright. The water was aerated.

By using high humidity, an attempt was made to vary the amount of top while keeping the water factor constant. Five plants in each lot were weighed at intervals and it was determined that

plants subjected to all treatments under the conditions of high relative humidity gained weight. The plants in the lower humidity responded similarly to those of previous experiments.

Roots began to appear by the third day and by the ninth day the maximum differences were attained (fig. 3). The high humidity seemed detrimental to plants with small tops (fig. 6, extreme right), but on the other hand it was possible to increase the amount of leafy top and still get maximum rooting. However, the untopped plants did not root quite as well as the ones with fewer leaves present. The response of untopped plants is important in considering the possibility of another factor, such as auxin which might be involved in the failure of rooting of untopped transplants. Smith (1944) showed that lateral buds on guayule transplants were inhibited in the presence of leaves and he suggested the possibility of inhibition of roots by the leaves also.

Girdling and relative humidity.—Differences in response between plants topped or girdled at a given level could largely be a reflection of the effect of water relations, and consequently, the substitution of girdling for topping should serve further to control the water factor while varying such effects of the leaves as might be brought about by substances translocated through the phloem. For an experiment of this sort one hundred and sixty plants were used. The same conditions of humidity were used as in the previous experiment. Four groups of twenty plants were used for each of the two conditions of humidity: In the first group, the plants were untopped, in the second, the stem was girdled above one cluster of leaves, in the third, the stem was girdled at $1\frac{1}{2}$ " above crown, and in the fourth, the plants were topped $1\frac{1}{2}$ " above the crown. In this manner any downwardly moving substances (in the phloem) would be delayed at the girdle but the girdled plants would have transpiring surfaces similar to those of untopped plants. The rooting response reached a maximum on the tenth day. The difference in response between humid and dry chambers was striking (fig. 4).

Under both conditions of humidity the girdled plants responded like untopped plants rather than plants topped at the level of the girdle. The significance of these responses is that rooting appeared to be limited primarily by water relations. The experiment was repeated with similar results (fig. 6). In the second trial the girdling treatment above one cluster of leaves was omitted.

The topped plant of the humid chamber shown at

40 to 100 per cent relative humidity, while the other three were kept at 95 to 100 per cent relative humidity, for a period of two weeks. Three topping treatments for each condition of humidity were used: The plants were untopped, girdled $1\frac{1}{2}$ inches above crown, or topped $1\frac{1}{2}$ inches above crown. All lateral roots present are newly formed.—Fig. 7. Untopped transplants with 30 to 36 inch root systems after three weeks in tap water; left, ungirdled; right, girdled six inches below crown. Note that girdled plant produced new roots, but only above girdle.—Fig. 8. Rooting responses of four-month-old unhardened and hardened transplants after two weeks in water. The two plants on the left were kept at 40 to 100 per cent relative humidity, the others at 95 to 100 per cent relative humidity. Note dried condition of the unhardened plant kept at the lower humidity. The small plants were stunted by the two months of hardening.

the right in figure 6 is typical of many that failed to grow. Microorganisms developed in masses over the roots, being concentrated during early stages near wounds where lateral roots had been removed. Some of the plants in the drier chamber also showed activity of microorganisms. Plants with short tops were more frequently affected but even untopped plants did not escape. In preliminary trials with the humid chamber it was found that when moisture was continuously sprayed on the foliage of untopped plants the roots nearly all became covered with slime. There seemed to be no actively parasitic organisms involved as necrosis of root tissues did not necessarily follow. A possible explanation is that the microorganisms thrived on organic substances which may have been leaching from the roots with greatly reduced transpiration streams.

Unhardened plants and relative humidity.—Unhardened guayule plants wilt within a short time after they are dug in the nursery, and field plantings of these plants are low in survival. To observe the rooting response of unhardened nursery stock when water was not limiting, the following experiment was performed. Four-months-old nursery plants of two types were used, instead of the stock of the previous experiments. One type, the unhardened plants, were grown for four months with fairly abundant watering (one inch per week in two applications). The other plants were treated in the same way during the first two months but were hardened by withholding irrigation during the second two months. These types were similar to some of those reported on by Kelley *et al.* (1945) and for which they give survival data for field transplanting trials.

Twelve hardened and twelve unhardened plants were used under each of the two conditions of humidity previously described. Within two weeks the unhardened plants had rooted in the humid chamber but not in the dry chamber, while the hardened ones grew under both sets of conditions (fig. 8). The succulent plants in the dry chamber wilted during the first day. After several more days the leaves and young stems were dead and brittle. Nearly all of these plants were somewhat shriveled at the end of two weeks. One, however, appeared to be more turgid than the rest and new roots were beginning to grow out. Possibly several more would have rooted eventually but the purpose of the experiment had been served: the unhardened leafy plants would generate new roots if transpiration was not excessive.

Girdling long root systems.—Another approach to the problem of rooting guayule transplants was by girdling tap roots six inches below the crowns. For this experiment the second season stock was again used. A pit was dug in the nursery and root systems were washed out to a depth of three feet. Forty plants with roots thirty to thirty-six inches long were obtained. These were placed in the outdoor wooden tanks containing aerated tap water. Lateral roots were removed from the tap roots to a

distance of six inches below the crowns. One-half of the plants were then girdled six inches below the crowns.

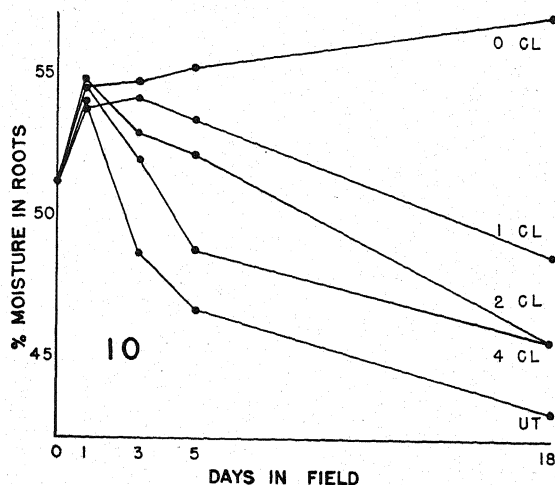
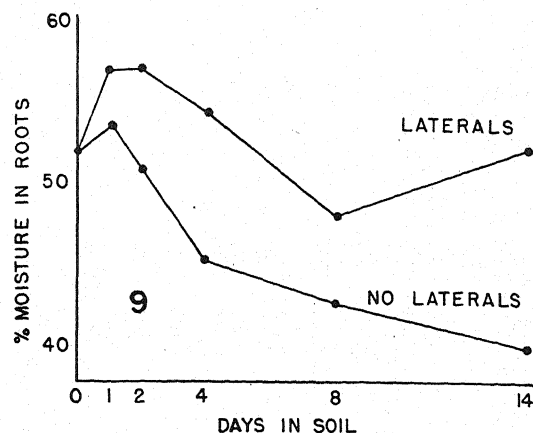


Fig. 9-10.—Fig. 9. Mean changes in moisture content of guayule tap roots after transplanting untopped plants with and without lateral roots. Each value is the mean of five plants.—Fig. 10. Mean moisture content, during eighteen days in the field, of tap roots of transplanted guayule stock with five degrees of defoliation. Each value is the mean of twenty plants. CL and UT denote number of clusters of leaves, and untopped, respectively.

New roots appeared in four days on some plants of both groups and by the eighth day the rooting was maximal: nineteen plants out of twenty rooted in both groups. In three weeks considerable new root growth had been made (fig. 7), but except for a few short thin roots below the girdles there was no indication of regeneration on this isolated part of the girdled plants. The ungirdled plants, however, produced new roots throughout the length of the root system.

The girdling of tap roots was tried on plants in the nursery with the result that new roots developed on the six-inch portion of the tap root above the

girdle under conditions provided by moist soil as well as in water.

While the results of the experiments in the humid chamber could be interpreted as indicating a factor besides water as being limiting in the generation of new roots on the untopped plants, such an interpretation would require further substantiation in view of the results of the present experiments on root girdling.

Effects of the presence or absence of lateral roots upon transplanting to soil.—A study was made of the effects of the presence or absence of lateral roots on untopped plants transplanted to the field. Eighty-five plants with many lateral roots were selected for this experiment on October 3. Five were taken at random for determination of moisture in leaves, stems, and roots (laterals removed). The remainder was randomized into two equal groups. In one group the lateral roots were removed and in the other they were left on. The plants were set out in the field in rows one foot apart with plants spaced at six inches in moist, sandy soil. One thorough furrow irrigation was given immediately. The next morning a sample of five plants was taken from each group for moisture determination. Additional moisture determinations were made on the second, fourth, eighth, and fourteenth mornings. On the eighth day new root tips were observed on three out of five plants with lateral roots. On the fourteenth day a total of forty plants was examined for new roots. None was found in the case of plants without laterals; thirteen out of twenty had new roots in the case of plants with laterals. Correlated with the rooting response was the moisture content.

The morning following the planting, the moisture content of both groups was higher than it was initially (fig. 9). On the second morning the plants with laterals were unchanged while those without laterals had already decreased considerably in moisture content. The difference between means at this point exceeded the one per cent level of probability. Subsequently the difference in moisture content between plants subjected to the treatments remained highly significant. These differences appeared prior to the appearance of new root tips. The effects of rooting on moisture content in the plants were not conspicuous until the last harvest when the plants with laterals were rapidly gaining in moisture content while the non-rooted plants of the other group were still desiccating.

From this experiment it may be concluded that in soil the laterals beneficially increase the contact between plant and soil moisture and also initiate new roots for the establishment of the transplant.

Effects of leaf removal on moisture content and rooting of transplants in the soil.—In previous experiments it was shown that when the tap roots of transplants were in water culture the amount of leafy top had a considerable effect on changes in moisture content and rooting of the plants. To determine whether similar changes occurred when

transplants were planted in the field an experiment was initiated on October 13, using a total of four hundred and twenty plants. At the time of preparation, twenty plants were taken at random for initial moisture content. The remaining plants were randomized into five groups for topping treatments which differed with respect to the numbers of clusters of leaves left on the plants: 0, 1, 2, 4, or all being present (untopped). The plants were set out in the field in rows one foot apart with plants spaced at four inches. Each replication consisted of five plants. Four harvests were made, each in the early morning. The first was made on the day after planting and the others on the third, fifth, and eighteenth days. Four replicates of five plants each were used in determining separately the moisture content of leaves, stems and roots.

The moisture contents of the roots increased uniformly for all treatments during the first night (fig. 10). By the third morning, variation in moisture content of roots as between treatments was highly significant, but no new roots were evident on the plants harvested. The plants harvested on the fifth day differed even more and while new roots were appearing at this time they were found on only the leafless plants (on ten out of twenty plants). On the eighteenth day new roots were found on plants subjected to the other treatments as well, but they were not abundant on the more leafy plants (table 4). The results of the rooting in the soil were closely parallel with those previously obtained in water.

TABLE 4. *Effects of presence of leaves on rooting, bud expansion, and moisture content of guayule plants 18 days in field (each value is the mean of 20 plants).*

Topping treatment	% rooted	% sprouted	% moisture content		
			Leaves	Stems	Tap root
0 Cl	85	80	...	55.0	57.1
1 Cl	25	5	57.1	46.6	48.6
2 Cl	15	10	55.1	47.1	45.5
4 Cl	15	0	53.9	43.9	45.5
UT	5	0	51.6	42.6	43.0
Initial moisture.	60.3	48.7	51.2

Bud sprouts were also counted on the eighteenth day and it is interesting to note that in several cases new roots were present before bud expansion could be detected (table 4).

In another experiment a direct comparison was made between greenhouse and field conditions using eight degrees of topping (fig. 5). In this experiment, also, new roots were present on many plants before bud growth was evident, both under water culture and soil conditions. A similar situation was observed in the case of plants which had remained dormant in the field for one whole year. Untopped plants had been planted in December of 1943 and some of them failed to resume growth until after the rainy season began during the fall of 1944. Drought during the intervening summer had re-

sulted in the death of all the leaves and even some of the stem tips of these untopped plants. When they were examined at the end of December in 1944 bud growth was not yet evident on many of the plants but new roots were present on all of those examined.

Under some conditions (*e.g.*, in the high humidity chamber in the greenhouse) bud expansion was observed before new roots were visible.

DISCUSSION.—Loomis (1925) states that for the vegetable plants he studied recovery from transplanting was affected by no factor which did not appear to be based, in its final effect, upon a change in the water supply of the plant. He segregated several vegetables into three groups based on the ease of transplanting and correlated this response with the ability of the plants to produce new roots. He concluded that the rate of new root formation was the most important consideration in the re-establishment of transplanted vegetables.

In the present investigation the data indicated that one of the most important effects of topping guayule transplants was to reduce the transpiring surface and thus to help prevent water from becoming the limiting factor. When net water loss was not prevented in leafy transplants, it was found that the moisture content of the tap roots frequently decreased to values below those which appeared to be critical for the formation of new roots. This occurred in less time than was required for the appearance of new roots under the best conditions attained. Since the formation of new roots is considered to be the critical factor in the establishment of guayule transplants, it becomes evident that water relations are of major importance. Further investigation is needed to understand why the moisture content of tap roots of leafy plants decreased so greatly even when the roots were suspended in water.

Since it is doubtful whether any root tips survive the procedures ordinarily used in transplanting guayule commercially, new root tips must emerge from those portions of the root system transferred. The variation in length and number of lateral roots on the transplants makes it important that the plant be able to initiate new root tips on about six inches of tap root alone. Any laterals present offer additional assurance of quick recovery.

Other effects of topping were described by Smith (1944) in experiments demonstrating hormonal inhibition in guayule transplants. He suggested that the inhibitor in some cases prevented new root growth. The benefit of topping, according to this interpretation, was to remove the major source of the inhibitor, the leaves. Smith (1945) subsequently determined the inhibitor to be auxin. It is known (Went and Thimann, 1937) that auxin can both initiate roots and inhibit their growth so it is possible that there could be a hormone mechanism inhibiting root formation in guayule. However, when stems of untopped plants were girdled below the leaves, no great improvement in rooting was found,

which is not what might be expected if the major limiting factor were an inhibiting substance descending in the phloem. On the other hand, untopped plants showed rooting in larger numbers when the relative humidity was increased, which is what might be expected if water were severely limiting. This evidence does not exclude the possibility of hormonal inhibition of new roots because 100 per cent rooting did not occur under highly humid conditions, but it demonstrates that the water factor in guayule transplants is of considerable practical importance. The results obtained in the experiments in which long root systems were girdled did not substantiate the possibility of hormonal inhibition of new roots.

At the present time insufficient data are available to clarify completely the problem of new root growth in untopped guayule transplants.

SUMMARY

It was found in a study of water relations of guayule transplants that the principal physiological benefit of topping came from reduction of transpiration. Without this reduction, tap roots of leafy transplants with few or no lateral roots almost always became low in moisture content and failed to generate new roots within a reasonable period of time, as contrasted with the best results obtained for certain degrees of topping, under conditions of both water culture and soil.

Changes in water content of the plants were studied by determining changes in fresh weights of individual plants in water culture and by harvesting randomized and replicated samples of field plantings for a determination of moisture content. Comparative measurements were made of water uptake and rate of transpiration for transplants with several degrees of topping, after they were placed in water culture.

Experiments on relative humidity and girdling were used to verify the validity of the relation between water content and the generation of new roots. It was found that when the relative humidity was high (95 to 100 per cent) little or no defoliation was necessary to obtain rooting of plants in water. The experiments on girdling did not completely exclude the possibility of hormonal inhibition of new roots on guayule transplants by leafy tops, but indicated that in leafy transplants water is frequently severely limiting in the formation of new roots.

SPECIAL GUAYULE RESEARCH PROJECT,
BUREAU OF PLANT INDUSTRY, SOILS AND AGRICULTURAL
ENGINEERING,
AGRICULTURAL RESEARCH ADMINISTRATION,
UNITED STATES DEPARTMENT OF AGRICULTURE,
SALINAS, CALIFORNIA

LITERATURE CITED

BAILEY, L. H. 1939. The standard cyclopedia of horticulture. Macmillan. New York.

- HAYES, H. K., AND F. R. IMMER. 1942. Methods of plant breeding. McGraw-Hill. New York.
- KELLEY, O. J., A. S. HUNTER, AND C. H. HOBBS. 1945. The effect of moisture stress on nursery grown guayule with respect to the amount and type of growth and growth response on transplanting. Jour. Amer. Soc. Agron. 37: 194-216.
- KRAUS, JAMES E. 1942. Effects of partial defoliation at transplanting time on subsequent growth and yield of lettuce, cauliflower, celery, peppers, and onions. U. S. Dept. Agric. Tech. Bull. No. 829. 35 pp., illus.
- LLOYD, F. E. 1911. Guayule, a rubber plant of the Chihuahuan desert. Carnegie Inst. of Wash. 213 pp., illus.
- LOOMIS, W. E. 1925. Studies in the transplanting of vegetable plants. N. Y. (Cornell) Univ. Agric. Expt. Sta. Mem. 87. 65 pp., illus.
- SMITH, PAUL F. 1944. Inhibition of growth in guayule as affected by topping and defoliation. Amer. Jour. Bot. 31: 328-336.
- SMITH, PAUL F. 1945. Auxin in leaves and its inhibitory effect on bud growth in guayule. Amer. Jour. Bot. 32: 270-276.
- SNEDECOR, G. W. 1940. Statistical methods. Iowa State College Press, Ames, Iowa.
- WEAVER, J. W., AND F. E. CLEMENTS. 1929. Plant ecology. McGraw-Hill. New York.
- WENT, F. W., AND K. V. THIMANN. 1937. Phytohormones. Macmillan. New York.

PLANT GROWTH UNDER CONTROLLED CONDITIONS. VI. COMPARISON BETWEEN FIELD AND AIR-CONDITIONED GREENHOUSE CULTURE OF TOMATOES ¹

F. W. Went and Lloyd Cosper

IN PREVIOUS papers of this series (Went, 1944a, 1944b, 1945b) the growth of tomato plants under rigidly controlled greenhouse conditions was described, and the relative importance of various external and internal factors involved in growth and fruit set was determined. Fruit set was abundant only at night temperatures between 15° and 20°C., under which conditions stem elongation was also optimal. Less growth and much less fruiting occurred at either higher or lower night temperatures. This temperature effect was much greater than that of relative humidity of the air, length of day or day temperature.

It seemed of interest to investigate whether under field conditions the night temperature played an equally controlling role in the development of a tomato plant. Since all environmental factors are fluctuating irregularly in a field experiment, only a correlational analysis of the effects of any one of these factors can be made, and this is only permissible when a large enough range of environments has been sampled. This was partially accomplished by growing five tomato varieties, which had been investigated in the air-conditioned greenhouses (Went, 1945b), in different localities in Southern California during 1944. More variations in climate were obtained by making plantings at monthly intervals, at each locality, so that essentially each planting was subjected to a different climate. The plants were watered and fertilized according to their needs, not according to a fixed schedule, which was precluded through the differences in climate and soil. Inspection of the field plots in the beginning of September, 1944, showed in general an excellent stand of tomatoes.

Table 1 shows the location of the field plots, their general treatment and appearance, and the type of records kept. The authors are especially indebted

to Dr. T. Whitaker for the La Jolla data, Dr. J. W. Lesley for the Riverside data, Dr. G. Mehlquist for the Westwood data, Mr. D. Buchrig for the Santa Monica data, and Mr. R. Dorland and Mrs. B. D. Ussher for the Temecula data. A total number of 900 plants was observed in the field plantings and over 10,000 fruits were harvested and weighed, adding up to a total of 1.5 tons of fruit.

EXPERIMENTAL.—Five tomato varieties, commonly grown in Southern California and representing different types, were selected for the main experiment. The varieties were: *Earliana*—first early home- and market-garden variety. Plant usually small, numerous flowers per cluster. *Marglobe*—midseason all-purpose tomato. Plant medium, dense foliage. *Pearson*—midseason Western variety. Plant small, growth determinate. *Improved Stone*—late canning variety. Plant large, dense foliage. *Pink Beefsteak*—late home-garden variety. Plant large, unfruitful. In addition a few plants of the following varieties were grown: *Illinois T 19*—Midwest early variety, resembles *Earliana*. *Large Late Red*—late local variety. Plant large. *Valley Giant*—late local variety. Plant large. All plants were raised from the same seed supply. The seedlings were kept in the greenhouse, and about one month after germination they were planted in the field, or in one-gallon crocks in coarse gravel to be subjected to temperature treatment in air-conditioned greenhouses. The latter have been described in an earlier paper (Went, 1945b). Each field planting consisted of six to ten plants per variety.

The greatest variable in the tests, disease, could be more or less eliminated by excluding all seriously diseased plants. In two localities (Temecula and La Jolla) practically no disease occurred. In Riverside and Westwood disease was not serious. Two localities (one in Pasadena, the other San Marino) had to be given up completely due to virus

¹ Received for publication July 14, 1945.

TABLE I.

Locality	Height above sea level	Soil condition	Plants trained to	Condition of plants	Records
Pasadena	A 800 ft.	Adobe, well worked	One stem	Fair, later much virus	Growth rate, production
	B 800 ft.	Adobe, very heavy	One stem	Very poor, later much virus	Growth rate, production
	C 800 ft.	Adobe, very heavy	One stem	Very poor, later much virus	No records kept
Westwood	200 ft.	Yolo clay	Not pruned	Good	No records kept
Altadena	1200 ft.	Very porous decomposed granite ^a	One stem	Fair, later virus and nematodes	Growth rate, production
Riverside	1100 ft.	Ramona sandy loam	One stem	Good, little disease	Growth rate, production
Temecula	1500 ft.	Adobe with much humus	Not pruned	Excellent, no disease	Growth rate, production
La Jolla	400 ft.	Sandy loam	Not pruned	Excellent, no disease	Growth rate, production
Santa Monica	Good, some virus	General conditions, only for first planting

^a 10 tons/acre decomposed manure. Basin irrigation.

diseases. In the other three localities (Santa Monica, Altadena and Pasadena) seriously diseased plants (especially with spotted wilt and curly top) were removed as soon as the symptoms appeared, and these plants were excluded from the calculations. Also, the second and third plantings in Altadena, which were seriously attacked by nematodes, had to be excluded. Proof of the fact that the tomatoes of the second planting in Altadena were not up to standard is found in table 4. Whereas the fruit weight in the first planting was comparable with that in the other localities, in the second planting it was only one-third as great.

RATE OF STEM ELONGATION.—Since the stem growth rates were most extensively investigated under controlled conditions, the first comparison will be made between the stem growth rates of plants in the greenhouse and in the field. Under controlled conditions it was found that whereas day temperatures had some effect, the most striking effect on growth in length was exerted by the temperature during night (Went, 1944a, 1945b). To make a comparison between the field and the greenhouse experiments, the growth outside was measured at approximately weekly intervals. Only the data on the plants trimmed to one stem are comparable with the greenhouse experiments and will be presented. The stem elongation rates of the

untrimmed tomatoes were about two-thirds as great as of the trimmed plants during the early stages of growth, and later decreased yet more.

During the early growth of the tomato plants their stem elongation rates are not constant, but after they have reached a length of about 50 cm., they have a constant growth rate under constant growing conditions. Therefore, only growth rates of plants 50 cm. and over have been included.

In earlier work (Went, 1944a) it was found that under optimal growing conditions fruit growth does not compete with vegetative growth, but that under suboptimal conditions, especially night temperatures above 18°C., the often described inhibition of vegetative growth by fruit development occurs. In the field all growing conditions are only seldom optimal, so that usually some decrease in rate of stem elongation is found when fruit set is heavy. To get away from this complication as much as possible, the growth rates of all plants of the different plantings were plotted separately, and a separate curve was then plotted for each variety, connecting the fastest growth rates for each measuring period. Usually there were one or two points where the rates of successive plantings almost coincided, when the rates of the older planting had not decreased as yet due to heavy fruit production, and the rate of the younger planting had just reached

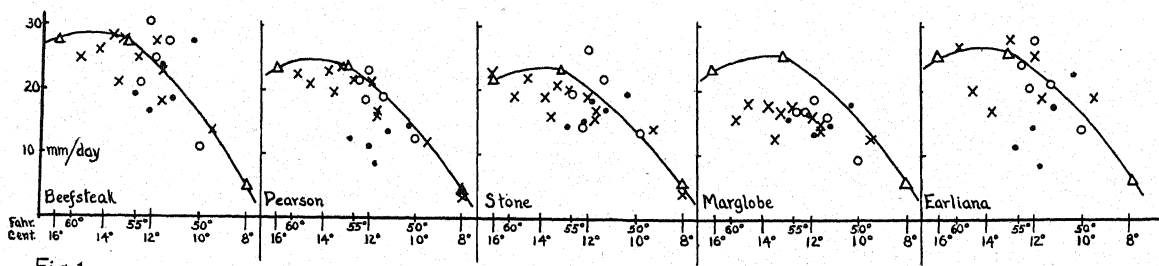


Fig. 1

Fig. 1. Growth rates (ordinates in mm. stem elongation per day) of five tomato varieties, as a function of the night temperature (abscissa). Triangles and drawn line: growth rates as measured in air-conditioned greenhouses. Crosses, circles and dots: growth rates as observed in the field (plotting daily minimal temperatures on abscissa). Crosses, growth rates as observed in Pasadena; circles, in Altadena; dots, in Riverside.

its steady rate. This derived growth curve, connecting the points of fastest growth, was used to plot figure 1.

For each period over which the growth was measured, the mean minimal and maximal temperatures were calculated, and then the growth rate was plotted as a function of the mean minimal or maximal temperature. Since no correlation between maximal temperature and growth was found, only the correlation with minimal temperature is shown in figure 1. In these graphs the growth rates of the same varieties, grown under controlled conditions, are shown as curves drawn through three points (data from Went, 1945b). Except for the *Marglobe* tomato the growth rates as measured in the field closely follow the growth rates of the same varieties as grown under controlled conditions. To show this it is necessary to plot growth against either the mean minimal temperatures for the field plants or the constant night temperature for the greenhouse plants. Even without further corrections for possible modifying factors (day temperatures, soil moisture, nutrition, length of photoperiod) such a close correlation is found as in figure 1. Therefore, it is safe to conclude that under field conditions the night temperature, as measured by the daily minimum temperature, is the most important single factor controlling stem elongation in tomatoes.

There is a consistent, though statistically insignificant, higher growth rate in the greenhouse than in the field, but this difference is about the same for all varieties except *Marglobe*. The varieties growing fastest in the greenhouse, also grow fastest in the field. The difference in rate of stem elongation in both the greenhouse and the field between the fast growing *Beefsteak* and the slower growing *Pearson* and *Stone* is highly significant. This all shows that the greenhouse experiments give an accurate account of the stem elongation responses of the tomatoes as observed in the field, with the exception of the *Marglobe* tomato.

It is also clear that the growth rates of the tomatoes growing in Riverside, Altadena and Pasadena were the same as long as the minimum temperatures were similar, so that other factors like soil, treatment, sunshine, etc., were of only secondary importance in regulating the growth rate. Night temperature is the controlling factor. This quantitative relationship holds, of course, only for healthy plants. The second and third field plantings in Altadena were heavily infected with nematodes, which reduced the growth rates well below those of the first planting. Therefore, only the Altadena data for the first planting have been included in figure 1. As soon as plants became infected with virus, especially with curly top and spotted wilt, growth rates decreased sharply, and such plants were removed and not included in the average growth rate.

Some of the growth rates of the plants at Riverside seem to lie below those at Pasadena and Altadena, especially in case of the *Pearson* tomato. This

is due to the longer measuring intervals at Riverside (once monthly). Since the *Pearson* has determinate growth, stem elongation ceases at some time when a height of about one meter has been reached. At shorter measuring intervals this growth stoppage can be detected earlier, and those plants can be left out of the calculation of growth rates. With the monthly measuring intervals this is impossible, and some plants which stopped growth were included among the growing plants, lowering the apparent growth rate.

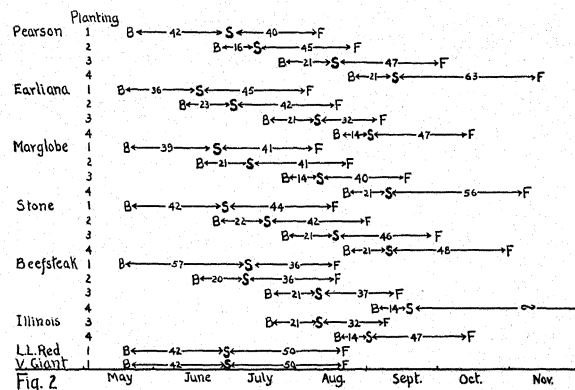


Fig. 2. First blossoming (B), first fruit set (S) and first ripe fruit (F) as observed in Temecula for eight varieties and four plantings. (For further information concerning planting dates see table 2.) Numbers refer to interval in days between blossoming, fruit set and fruit ripening.

A similar explanation holds for the *Earliana* tomato. Although this variety does not have determinate growth, its early fruit set is usually so heavy that it soon interferes with vegetative growth, which is resumed at a normal rate only after the first crop of fruits has ripened. This retardation of growth also is detected only for short measuring intervals.

The discrepancy of growth rates in the greenhouse and in the field for the *Marglobe* tomato needs some further discussion. The drawn curves are all taken from the data of a single experiment in the greenhouse, published as table 3 in Went (1945). Due to several reasons (poor germination, disease) only one or two *Marglobe* tomato plants were left for each treatment, and this may have caused the values to be too high. In a subsequent experiment (table 4, Went, 1945b) the rate of stem elongation of the *Marglobe* tomato was well below 20 mm./day for the 13° night temperature, in the same range as the field experiments. This latter experiment was representative of other varieties, since at 13° night temperature the *Norton Stone* tomato grew 24 mm./day. In another experiment (not published) the *Stone* tomato grew 23 mm./day at 16° night temperature, whereas in figure 1 those values for the *Stone* are 23 and 22 mm./day. Therefore, it is likely that the growth rates of the *Marglobe* in the greenhouse experiments of figure 1 are too high.

BLOSSOMING AND FRUIT SET.—In only two of the field experiments was the date of first open flowers and setting of fruit noted. The data for one of these are shown in figure 2. It appears that there is a constant lapse of time between sowing and opening of the first flowers. This is also observed in the greenhouse, where the first flower cluster appears always at about the same node, and where the number of nodes formed in unit time is not greatly influenced by night temperatures above 10°C . There is, however, a great variation in the length of time between the opening of the first flower and the setting of the first fruit. Within one variety this may vary between 20 and 57 days, or between 16 and 42 days. During the summer months this period is about constant, but in spring and fall, when the night temperatures are too low for the setting of fruit, no set is possible before a period of favorable night temperature occurs. In this way the first setting in the first and second planting was almost simultaneous in the *Beefsteak* tomato (fig. 2). Under all conditions and in all localities every plant flowered normally; great differences were found in fruit set as evidenced by fruit production.

FRUIT PRODUCTION.—As figure 2 indicates, a fairly constant time elapses between the first visible setting of fruits and their final ripening. This period lengthens for the last plantings, as the growing

conditions for the fruit become unfavorable. These data do not show the length of ripening period for the individual fruits, since the flowers were not tagged, but they give a general idea of the ripening period, which is not very different for the various varieties.

Figure 3 gives a general idea of the fruit production in the different localities. It shows the production of ripe fruit per plant as a mean of all varieties for each planting up to the end of November. The small amount of fruit ripened after that period in all localities except La Jolla has been neglected, also the unripe fruit is not included. The outstanding results are: the first plantings showed approximately the same production in LaJolla and Temecula, where the plants were not pruned. In Riverside, Pasadena and Altadena, where the plants were all trimmed to one stem, the production was much less, but yields in the three localities were of the same order of magnitude. With only a few exceptions, the production rate of each variety in each locality fell off sharply for each successive planting. This drop in production was much less in La Jolla than in the other localities, and the third and fourth plantings there were both fairly productive (about 50 per cent of the first planting).

The varieties showed consistent trends in all localities. In the last productive planting the

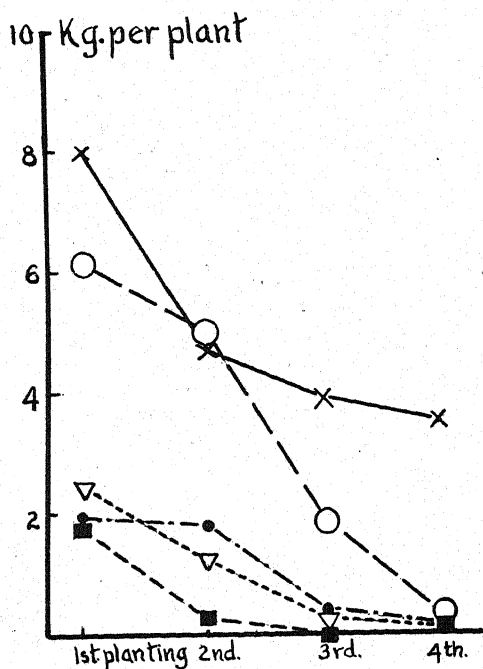


Fig. 3

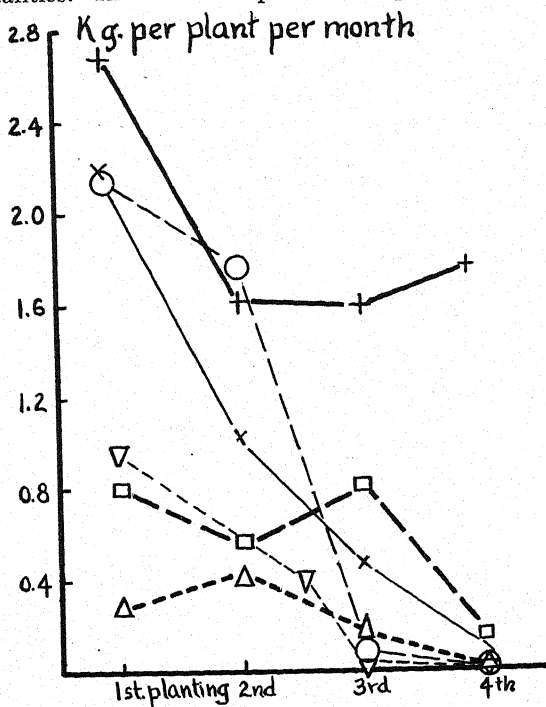


Fig. 4

Fig. 3 and 4.—Fig. 3. Total fruit production per plant of each of the four plantings as a mean of all varieties for five localities. Crosses, La Jolla; circles, Temecula; triangles, Riverside; dots, Pasadena; squares, Altadena.—Fig. 4. Total fruit production per month and per plant (mean of all varieties) for each of the four plantings in three localities. Values for August and September are shown with thin lines, for October and November with heavy lines. Crosses and plusses: early and late harvests in La Jolla. Circles and squares: production in Temecula. Inverted and upright triangles: early and late harvests in Riverside.

Earliana was consistently better than the other varieties. This is connected with the property of *Earliana* to set fruit sooner and at lower temperatures than the others. Whereas the *Stone* made a poor showing in the first planting in all localities, in the second planting it did absolutely or relatively better. In the first and third plantings the *Pearson* was superior in productivity. *Beefsteak* was poor especially where the night temperatures were low.

The data for the production of ripe fruit as collected at the different localities are fairly detailed, since once or twice each week all ripe tomatoes, both market size and small ones, were picked and weighed and recorded per plant. Figure 4 shows a breakdown of production for each locality and each planting according to early and late tomatoes. The fruit collected in August and September, when the plants started to come into production, is shown in the curves with the thin lines. These data show for each locality a rapid and steady decrease in fruit production, as could be expected. The data for October and November production (heavy lines) are very different; once the plants have reached their maximal rate of fruit production, they continue to produce at a rate which is rather independent of the time of planting. The values for the fourth planting in Temecula and Riverside are too low due to the fact that in these late plantings, the tomato plants never reached sufficient size for proper production, at the prevailing low night temperatures.

The data as presented in figures 3 and 4 do not allow an analysis of fruit production as a function of climate. As a first step in such an analysis graphs were prepared, in which for each locality the maximum and minimum temperatures were plotted against time. In those same graphs the production of each variety for each planting was recorded as grams of ripe fruit produced per plant per day. Figure 5 presents part of these records showing (confirmed by the curves of the other varieties and localities, not presented here) that great variations in the ripening occur. At some periods five to ten times as much fruit ripens as in preceding or succeeding weeks.

Usually one or two weeks after a plant produced its first fruit a heavy crop is harvested, then a marked drop occurs, followed by one or more waves of fruit production. This behavior is partly due to the innate response of the tomato plant. In the few cases when tomato plants were kept in the air-conditioned greenhouses beyond the period of first fruit production, ripening of fruits took place as shown in figure 6. After an initial high rate, when the fruits on the first flower cluster ripened almost simultaneously, a drop and new rise occurred two weeks later, when the fruits of the second cluster ripened, and after that production evened out to a more steady rate. This happens when the temperature has been controlled throughout the setting and ripening period. Therefore, the first rise and drop in fruit production in the field experiments can be

accounted for by the simultaneous ripening of the fruits on the first flower clusters. The later fluctuations in the field plantings are much more pronounced than those in the greenhouse, and therefore presumably have another cause. This cause must be an external one, acting similarly on all plants and varieties, since most of them show maxima and minima in production at the same time, irrespective of planting date (see fig. 5).

FRUIT RIPENING AND DAY TEMPERATURE.—In the greenhouse experiments it was found that at 17° as well as at 20° and 26° day temperature fruit development is normal at the proper night temperature, and that development is somewhat slower at the lower day temperature. In the field experiments no indications were found that day temperature had any effect on fruit production. In La Jolla the maximum (day) temperature during the early setting period (August) hovered between 20° and 24°. During October fruit production kept up very well, but maximum temperatures varied between 16° and 20°. In Pasadena, Altadena, Temecula and Riverside maximum temperatures during August varied between 30° and 40°, and in October they still ranged between 25° and 35°, whereas fruit production was well below that in La Jolla. Extra warm or cool day temperatures were not correlated with production maxima or minima, and the hot spells only caused sunburn of the exposed tomato fruits, without interrupting their ripening.

FRUIT PRODUCTION AND NIGHT TEMPERATURE.—A comparison between the production and temperature in La Jolla and Temecula shows that during July and August the minimum night temperatures are on an average only 2° lower in Temecula, but in October and November they are 3° to 8° lower. Fruit production in La Jolla remained fairly constant through October and November, whereas in Temecula the decreased night temperature (below 12°) was paralleled by a marked decrease in fruit production. The same drop in minimum temperature and concomitant drop in production was found in Pasadena, Riverside and Altadena. This already gives a strong indication that in the field as in the greenhouse (Went, 1944a), tomato fruit set and production occur predominantly at night temperatures above 14°.

To allow a closer analysis of this phenomenon, the relation between fruit production and night temperatures preceding fruit ripening was investigated. Since such a marked fluctuation exists in the amount of fruit ripening each week, it might be expected that the maxima in fruit production are the results of favorable conditions sometime earlier. If this is true, the same favorable night temperatures should have occurred before each maximum in fruit production. By averaging the temperatures as they occurred on each successive night before each maximal production, the accidental fluctuations should cancel out, leaving only the essential fluctuations which determined fruit ripening. The calculations were started commencing with the tempera-

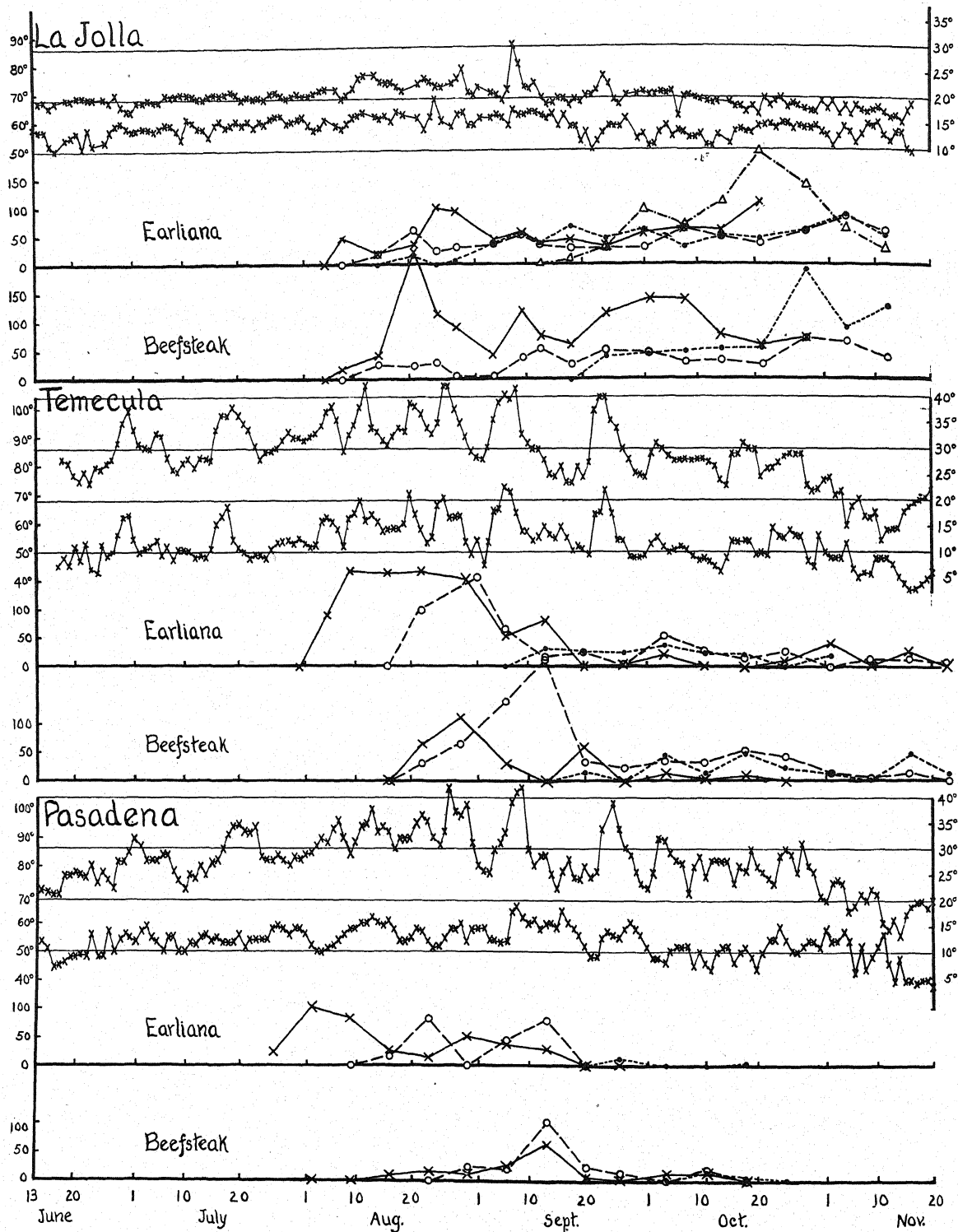


Fig. 5

Fig. 5. Daily maximum and minimum temperatures in La Jolla, Temecula and Pasadena for the period of June 13 to November 20, 1944. For each of these localities the production of *Earliana* and *Beefsteak* tomatoes is shown in grams of ripe fruit formed per day per plant. Crosses and solid lines, first planting; circles and broken lines, second planting; dots and dotted lines, third planting; triangles and dot-dash lines, fourth planting.

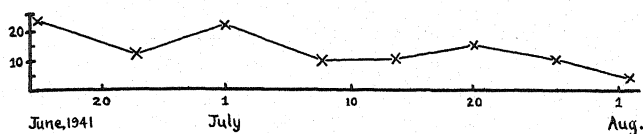


Fig. 6

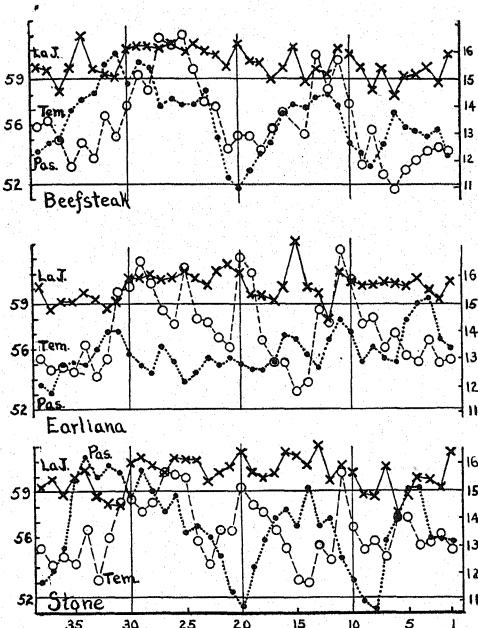


Fig. 7

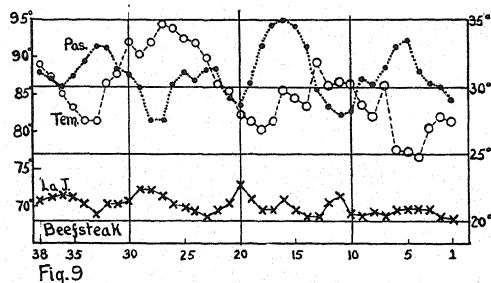


Fig. 9

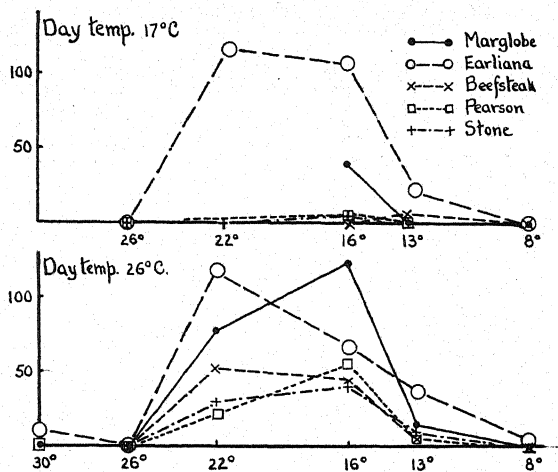


Fig. 8

Fig. 6-9.—Fig. 6. Fruit production in grams of ripe fruit per plant per day for San Jose Canner tomato, grown in air-conditioned greenhouse at 26°C. during day from 8:00 until 16:00 and 20° during night (16:00 until 8:00).—Fig. 7. Mean daily minimal temperatures (ordinates, at left in degrees Fahrenheit, at right in degrees Centigrade), occurring 38 to 1 days before a maximum in fruit production in three localities for three varieties. Crosses and solid lines, La Jolla; circles and broken lines, Temecula; dots and dotted lines, Pasadena.—Fig. 8. Fruit production in grams per plant for five varieties grown at two day temperatures and six night temperatures in air-conditioned greenhouses (data from Went, 1945). Dots and solid lines, *Marglobe* tomato; circles and broken lines, *Earliana*; squares and dotted lines, *Pearson*; crosses and broken lines, *Beefsteak*; plusses and dot-dash lines, *Stone*.—Fig. 9. Mean daily maximal temperatures (ordinates at left, degrees Fahrenheit; at right, degrees Centigrade), occurring 38 to 1 days before a maximum in fruit production for the *Beefsteak* tomato, in La Jolla (crosses), Temecula (circles) and Pasadena (dots).

ture on the 38th night before each maximal production, and continued to the night before the maximum. Only the data for August, September and October production were used, since the later production became much smaller, and the lower night temperatures increased the ripening time. For each of three localities (Pasadena, La Jolla, Temecula) the night temperatures preceding fruit ripening were averaged separately for each variety. Usually about three production maxima for each of the three first plantings were averaged, which makes each curve an average of from six to twelve individual cases.

Figure 7 shows the results plotted graphically for three varieties. First the curves for the *Beefsteak* tomato will be considered. In Pasadena the mean minimum temperature 38 days before maximum fruit production was about 12°C., increasing rapidly to 16°C., 31 days before ripening, hovering between 15° and 16° for five days, and then decreasing again. A second maximum was reached

twelve days before ripening, but this reached only 14°C. In Temecula practically the same sequence of temperatures was found, from a low of 12°C., the minimum temperatures increased to 15° to 17° for a period of six days, 24 to 29 days before ripening; a secondary maximum of about 15° occurred 11 to 13 days before ripening. Both curves show a marked maximum of 15° or higher, for at least five successive nights around 30 days before maximum fruit production.

Production was calculated only on a weekly basis and was averaged on a fruit weight produced per day per plant. But since it was plotted for the week preceding the actual picking, the time lapse between observed favorable temperatures and fruit ripening might be as much as four days longer than shown in figure 7. Therefore the 30 days shown in figure 7 for the *Beefsteak* tomato is probably actually longer, and consequently it coincides well with the 36-day lapse of time between fruit set and

ripening in the *Beefsteak* tomato as shown in figure 2. This, in combination with the greenhouse experiments, indicates that when a period of at least five days of minimum temperatures of 15°C. or over occurs, a maximum in the production curve of ripe *Beefsteak* tomatoes can be expected a month later. Apparently the *Beefsteak* tomato does not set fruit when the night temperatures fall below 15°C. This is corroborated by figure 8, where the critical temperature for *Beefsteak* fruiting was found to be between 13° and 16°.

In La Jolla the minimum temperatures hovered around 16°C. all the time, and consequently the *Beefsteak* tomatoes could set fruit almost continuously, and less fluctuation in fruit production was found in that locality than in Temecula or Pasadena. The curve of mean minimum temperatures in the 38 days preceding maximum fruit production in La Jolla therefore is flat without pronounced minima or maxima. How well fruit production on *Beefsteak* tomatoes can be used as a minimum thermometer is shown by the fact that on September 2 visual inspection of the field experiment at La Jolla led to the conclusion that night temperatures there presumably had been 3°C. higher than in Pasadena. Actually the meteorological records showed that they had been 2.5° higher on an average for the previous month.

Figure 7 also shows the mean minimum temperatures on the 38 days preceding maximum fruit production in the *Stone* tomato. These curves are very much like those for the *Beefsteak* tomato. In Pasadena there was a period of 28 to 35 days before each maximum in fruit production, when the minimum temperatures were about 15°; in Temecula there was a similar period 25 to 27 days before picking. At no other periods before ripening were such high minimum temperatures reached.

In La Jolla again no such temperature correlations were found, due to the fact that minimum temperatures were high enough most of the time to insure good fruit set in *Stone* tomatoes. The greenhouse experiments again show that the critical night temperature for fruit set in the *Stone* tomato lies between 13° and 16° (fig. 8). In contrast with the *Beefsteak* and the *Stone* tomato the fruit set of *Earliana* was appreciable at 13° night temperature in the air-conditioned greenhouse, both at 17° and 26° day temperature (fig. 8).

The third set of curves of figure 7 shows that in the field the *Earliana* behaved entirely differently from the other two. In Pasadena the mean minimum temperatures before maximum fruit production remained under 14°C., for almost all of the 38 days, with no maximum between 25 and 35 days. In Temecula violent fluctuations occurred, with no single maximum which was higher or more extended than the others. Again in La Jolla there was no maximum of any importance. Since nevertheless in the *Earliana* the fluctuations in fruit production were very pronounced in the field, and the synchronization of ripening was not brought on by a

period of high minimum temperatures about a month earlier, the rhythm in production must be due to another climatic factor. Inspection of figure 7 suggests that five weeks before a ripening period low night temperatures usually prevailed, which suddenly became higher. Therefore, it might be suggested that, although *Earliana* can set fruit at temperatures below 13°, this is slow, so that any rise in temperature will accelerate setting and cause a maximum in fruit production a month later.

The fruit production of the field plots in Riverside and Altadena showed fluctuations similar to those in Pasadena and Temecula, and maxima in production of *Beefsteak* and *Stone* were in each case preceded by a period of minimum temperatures of 15°C., or higher, about one month before the picking dates. Since the production in Altadena was low for the second and third plantings due to nematode infection, and the fruits in Riverside were picked only once every two to four weeks, these data are less suitable for an analysis as shown in figure 7.

The *Pearson* and *Marglobe* tomatoes did not show such marked maxima in the mean minimum temperatures one month before picking as did the *Beefsteak* and the *Stone*, but at least in Pasadena there was a period of four days 30 to 35 days before maxima in production when night temperatures of 15° were reached. The Temecula curves for both *Pearson* and *Marglobe* resembled those for *Earliana* in the same locality. In the greenhouse these varieties were intermediate between *Earliana* on the one hand and *Beefsteak* and *Stone* on the other hand (fig. 8).

Figure 9 shows the same type of analysis as that of figure 7, only the mean of the maximum temperatures preceding each maximum in picking have been entered on the ordinate. It can be seen that for the *Beefsteak* tomato at no time did a range of temperatures occur accounting for fruit production which was different from preceding or succeeding periods, and which was approximately the same for Pasadena, Temecula and La Jolla.

In table 2, the time is indicated when fruit production became greater than 25 grams of ripe fruit per plant per day. The days are numbered consecutively, May 1 being 1 and June 1 being 32, etc. It is seen that for the second planting harvesting dates for all three localities were about the same for any one variety, showing that for that planting, fruiting conditions were about equally good everywhere. But for the first, third and especially the fourth planting La Jolla was far superior to the other localities.

The conclusions drawn from figures 7 and 8 are also corroborated by table 2. The tomato varieties which need the highest night temperatures for fruit set (*Beefsteak* and *Stone*), ripened almost simultaneously in the first and second planting in each of the localities. From figure 5 it is evident that they could not have produced earlier due to low minimum temperatures. *Earliana*, which sets fruit at the low-

TABLE 2. Date in days after May 1, 1945, when tomatoes were sown and planted, and when for the first time fruit production reached a rate of 25 gr. of ripe fruit per plant per day. May 1 is recorded as 1, June 1 as 32, July 1 as 62, etc. When by December 1 no appreciable fruit set was noted, harvesting date was set as >240.

Locality Planting	La Jolla					Temecula				Pasadena			
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th	1st	2nd	3rd	4th
Beefsteak	103	107	116	145	>240	110	114	(148)	>240	127	122	168	>240
Stone	108	115	141	164	>240	109	116	(162)	>240	124	125	(172)	>240
Large Late Red	110
Pearson	110	120	137	171	>240	103	116	151	(203)	110	125	169	>240
Marglobe	101	121	138	168	>240	98	115	143	193	112	124	177	>240
Valley Giant	103
Earliana	99	108	124	146	>240	92	110	135	195	87	109	>240	>240
Illinois T 19	146	186	>240	>240
Sown	-19	8	29	60	95	-28	1	34	61	-28	1	34	61
Planted in field	10	29	57	90	122	-6	21	73	101	-1	29	64	94

est night temperature, had the greatest difference in ripening date between the first two plantings, and *Pearson* and *Marglobe* were about intermediate in both respects. In La Jolla with its favorable summer climate for tomato fruiting, the third planting ripened relatively earlier than the second, and the fourth ripened just about one month later than the third. In Temecula the third planting produced about one month later than the second, and the fourth barely reached the production stage 1.5 to two months after the third. In Pasadena the third planting was already 1.5 months behind the second planting, and the fourth planting never produced more than 0.2 fruit per plant.

The data from table 2 can also be used to compare with the work of Boswell *et al.* (1933), the most extensive cooperative tomato growing experiment heretofore carried out. In this study, nine tomato varieties were grown in widely separated localities for three successive seasons, and the results were summarized in a table which gives, among other data, the time from transplanting to harvesting for each of their nine varieties. Three of these were tested in the present experiment. Table 3 shows how close the present results agree with those obtained more than a decade earlier. When the climatic conditions were favorable during the growing period (third and fourth planting in La Jolla, third planting in Temecula), harvesting times fall within the specified period typical of the variety. But the table also shows that for other climatic conditions, even though the total production may be much greater (earlier plantings) a

much longer time may elapse than is considered typical.

INDIVIDUAL FRUIT WEIGHT.—The data of Boswell *et al.* (1933) do not agree so well with ours as far as the fruit weight is concerned. For each variety they mention a typical weight, but they state that soil and climate greatly affect fruit size. By looking at table 4 and figure 10, it will be seen that only

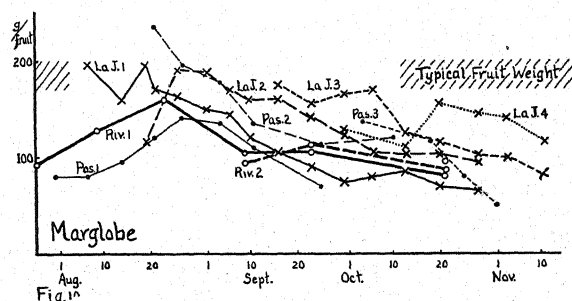


Fig. 10. Mean weight of individual fruits (ordinate, grams per fruit) of the *Marglobe* tomato as a function of the season (abscissa, dates). Typical fruit weight as published by Boswell *et al.* (1933). Solid lines, first planting; broken lines, second planting; dashed lines, third planting; dotted lines, fourth planting; heavy lines, Riverside data; thin lines, Pasadena data; intermediate lines, La Jolla data.

under special conditions can one speak of a typical fruit weight. Such conditions are defined for the tomatoes produced during the second week of production in the middle of the best fruit ripening season. Figure 10 shows how in La Jolla the weight

TABLE 3. Length of time in days between planting of tomatoes in field and fruit production exceeding 25 gr. fruit/plant/day. Data for La Jolla, Temecula and Pasadena taken from table 2, the "standard" data from Boswell *et al.*, 1933.

Planting period	La Jolla					Temecula				Pasadena				Standard (Boswell <i>et al.</i> , 1933)
	1	2	3	4	5	1	2	3	4	1	2	3	4	
Earliana	89	79	67	56	>118	98	89	62	94	88	80	>176	>146	55-65
Marglobe	91	92	81	78	>118	104	94	70	92	113	95	113	>146	70-80
Stone	98	86	84	74	>118	115	95	89	>139	125	96	92	>146	75-90

TABLE 4. Mean weight in grams per fruit for each planting in four localities. All fruits, also the culls, were weighed and averaged. Total number of fruits harvested in La Jolla, 3647; Riverside, 1475; Pasadena, 843; Altadena, 528. Typical fruit weight as published by Boswell et al. (1933).

Variety	Where planted	1st planting	2nd planting	3rd planting	4th planting	Typical fruit weight
<i>Beefsteak</i>	La Jolla	185	177	191	228
	Riverside	263	289	262
	Pasadena	170	197	269
	Altadena	198	67
<i>Stone</i>	La Jolla	92	146	83	135	195-215
	Riverside	92	140	144
	Pasadena	88	156	135
	Altadena	76	60
<i>Pearson</i>	La Jolla	125	123	146	224
	Riverside	125	120	155
	Pasadena	136	135	155
	Altadena	134	44
<i>Marglobe</i>	La Jolla	112	128	119	132	170-200
	Riverside	112	95	95
	Pasadena	117	141	90
	Altadena	104	34
<i>Earliana</i>	La Jolla	120	118	123	176	125-155
	Riverside	148	125	101
	Pasadena	113	70	74
	Altadena	94	33

of *Marglobe* tomatoes starts at a high level and then gradually drops to about one-half their typical weight. This is observed in all plantings, independent of the weather. All four curves follow each other at about three-week intervals.

In Pasadena and Riverside the early fruits produced were much lighter (or heavier) than the "typical" fruit weight, but in the course of a few weeks their size increased, and later closely paralleled the fruit weight in La Jolla for the same planting. The other varieties did not behave in the same way. Table 4 shows that the fruit produced in the fourth planting are unexpectedly large—heavier than the "typical fruit weight"—and are more than twice as heavy as those ripening simultaneously on the tomato plants of the earlier plantings. This greater size is typical of all the tomatoes planted last, as table 4 shows. This may be caused by the poorer conditions of fruit set (lower night temperatures) coupled with suitable conditions for fruit growth. In Pasadena and Riverside the individual fruit weight from week to week was much more variable than in La Jolla and fluctuated between wide margins. This variability is connected with growing conditions which fluctuated much more than in La Jolla.

In Santa Monica only the first planting was made. Total yield per plant was estimated and in table 5 these data are compared with those in La Jolla and Temecula, since in none of these localities were the plants pruned. Except for the *Marglobe* in Santa Monica and the *Beefsteak* in Temecula the yields are of the same order of magnitude. The climate of Santa Monica is much like that of La Jolla, night temperatures being about $\frac{1}{2}^{\circ}\text{C}$.

lower, day temperatures from 2° to 5° higher than in La Jolla.

TABLE 5. Tomato production for unpruned plants in kg per plant for the first planting. The Santa Monica weights were estimated.

Variety	Locality		
	Santa Monica	La Jolla	Temecula
<i>Beefsteak</i>	9	8	2
<i>Stone</i>	3	6	5
<i>Large Late Red</i>	10
<i>Pearson</i>	13	11	9
<i>Marglobe</i>	1	10	9
<i>Valley Giant</i>	7
<i>Earliana</i>	7	5	6

One important exception to the rule that fruit set is controlled by the night temperature was observed in Temecula. Towards the end of November when the night temperatures hardly reached 5°C ., fruit started to set generally on many plants. This occurred immediately after a rainy period with dark cloudy days. In some field experiments (unpublished) in which tomato plants were covered with muslin during late fall and winter, fruit set was observed to increase by decreasing the normal afternoon daylight intensity to 30 or 10 per cent. For this reason it seems permissible to consider the fruit set in Temecula at the end of November as a result of decreased light intensity during the rainy period, when part of the day was dark enough to become a functional night and effective for tomato fruit set (Went, 1945a). Therefore this observa-

tion is not necessarily in conflict with the conclusions reached concerning the limitation of fruiting by night temperatures.

TABLE 6. *Relative tastiness of tomatoes, ripe from the vine, as tested by various persons. 1 = Excellent flavor, sweetness and acidity. 6 = Insipid, lacking flavor.*

Locality	Temecula	Pasadena	Westwood
Date	Sept. 3	Sept. 7	Sept. 11
No. of persons testing	5	3	2
Variety:			
<i>Earliana</i>	1	1	1
<i>Large Late Red</i>	2	2	..
<i>Marglobe</i>	3	..	3
<i>Valley Giant</i>	3	3	..
<i>Beefsteak</i>	4	..	6
<i>Stone</i>	5	5	4
<i>Pearson</i>	6	6	4

THE TASTE OF DIFFERENT VARIETIES.—In different localities the tomatoes were tasted periodically, and direct comparisons could be made of the different varieties. Vine-ripened fruits were tasted by two to five persons and the varieties were graded from best to poorest. Usually there was complete agreement about the merits of the varieties between individual tasters, and table 6 summarizes the results. In later tests (not shown) it turned out that there was a seasonal change in tastiness. Whereas the early fruits of *Beefsteak* were considered very poor in most tests, later in the season *Beefsteak* was considered an excellent fruit, when it had become sweeter and tastier. *Pearson* also showed a marked improvement in taste in the later harvests. *Earliana* was consistently considered one of the best tasting varieties; it had an excellent blend of sweetness and acidity.

The increase in sweetness of fruits towards the end of the season can be explained as being based on the effects of temperature on sugar translocation (Went, 1944a). The lower the night temperature (at least as far down as 8°C.), the larger the amount of sugar translocated from the leaves. Since the growth processes are slowed down at the lower temperatures, the increased amount of sugar arriving in the fruits is not depleted by utilization for growth, and the fruit becomes much sweeter.

DISCUSSION.—With the definite information obtained on tomato growth and fruiting in the air-conditioned greenhouses (Went, 1944a, 1945b), it seemed worth while to investigate whether the same conditions also control growth in the field. Most information on the factors controlling tomato production in the field is rather indefinite, and many ambiguous statements are found, e.g., "thus, excessive vegetative growth may be a result as well as a cause of poor setting" (Work, 1942, p. 32), or the tomato "requires for even a fair yield very constantly favorable conditions" (Tracy, 1907).

Thompson (1939) claims for crops in general that "favorable climatic conditions are essential for successful production of crops . . . since high yields and good quality . . . cannot be obtained in regions having an unfavorable climate." Yet such "favorable" conditions or climates are seldom clearly defined. The most definite for tomatoes seems: "As more recent research has demonstrated, average temperatures as low as 50°F., and as high as 100°F., tend to prevent fruit setting and temperatures of 70° to 85°F., are more favorable" (Porter and MacGillivray, 1942).

In the present experiment tomatoes were planted at monthly intervals in the same locality, thereby being subjected to very different climates, while edaphic conditions were the same. In different localities the climates also varied, so that in all a fairly wide range of conditions was obtained, some favorable, some definitely unfavorable for fruiting of tomatoes. Analysis of the data made it possible to give a precise definition of the "favorable" conditions for tomato production in the field.

Although it is not claimed that the minimum daily temperature is the *only* factor involved in stem elongation and fruit production, doubtless the night temperature is the factor *controlling* tomato growth in the field. Not enough data were available to analyze the modifying effects of other factors such as day temperature upon fruiting but, especially from figure 1, it is clear that they modify only slightly the response to night temperature. This has considerable practical importance, for we can state now categorically that the mean temperature of a locality does not express its suitability for tomato culture. On the other hand, the minimum temperature, broken down in monthly or weekly averages, is an excellent guide for judging a prospective tomato growing region. This minimum temperature also allows a choice of the varieties most suitable for planting and selection of proper planting dates. By keeping a running record of minimum temperatures during the growing period, it is possible to predict a month ahead when heavy fruit production can be expected. This is of importance for marketing and canning operations.

The summer of 1944 was very cool, and in none of the localities did any prolonged periods of high minimal temperatures occur. Therefore, limitation of fruit production by too high night temperature, which was so marked in the greenhouse experiments (Went, 1944a, 1945b), was not encountered at all in these field tests. This condition may seriously interfere with tomato production in the tropics, the Southwest and certain interior desert regions, where night temperatures may not drop below 22° for periods of weeks or longer.

Another important fact was ascertained. The same factors controlling growth of tomatoes in the greenhouse were found to be operative in the field, and many growth responses were quantitatively the same in field and greenhouse. Since tomato experiments are completely reproducible under the con-

ditions which can be controlled in the air-conditioned greenhouses (Went, 1945b) experiments carried out in such air-conditioned greenhouses can be used instead of less reproducible field experiments. The latter have to be repeated year after year, since each year with its different sequence of temperatures causes a different response of the tomato plants.

Before many tomato field experiments can be omitted, accurate greenhouse experiments have to establish how long a favorable night temperature has to last to produce an effect and for how many successive days, and whether previous or subsequent higher or lower night temperatures affect the response, nullify or intensify the effect, and to what extent light inhibits this temperature response. Other experiments have to be carried out to find the interactions of night temperatures on the one hand and age of the plants, day-time conditions, cultural practices, nutrition, watering and soil temperatures on the other hand. In properly equipped air-conditioned greenhouses of sufficient size this can all be investigated in even a limited number of multi-factorial experiments, and absolutely accurate results can be obtained, valid at any time anywhere in the world. A beginning of this has been made (Went, 1944a, 1944b, 1945b).

The importance of accurate knowledge concerning the response of crop plants to their surroundings lies in: (1) establishing the validity of the causality principle under practical field conditions; (2) enabling the choice of the best localities, climatically speaking, for growing each variety of crop plant by merely consulting weather records, without resorting to years of field tests; (3) calculating whether the yield in a certain locality is what should be expected under the prevailing climatic conditions. This gives the grower a chance to check up on his efficiency, to improve his cultural practices if necessary, and to calculate loss due to diseases.

To prevent misunderstanding, it should be stressed that the effects of light intensity, length of day, nutrition, water, etc., can not be deduced from these field tests. This could not be done because not sufficient variation in these conditions existed in the experiments. Nutrients and water were abundant at all times, and did not limit development. Light intensity was high during most of the experimental period in all localities. Length of day also did not vary enough; apart from the differences to be expected from the temperature change in the course of summer and fall no other major factor seemed to influence the results.

SUMMARY

Five different tomato varieties were grown in eight different localities throughout Southern California. By making three to five separate plantings at monthly intervals, a further climatic differentia-

tion became possible. It was found that the growth rate of stem elongation, when plotted against mean minimal temperature during the measuring interval, closely followed the growth rate of the same variety in the greenhouse. This held irrespective of the locality or time of year, showing that night temperature was the main factor controlling stem growth rate.

Fruit production differed greatly according to variety, locality and time of planting. Analysis of the production data showed no correlation between maximum temperatures and the amount of fruit ripening.

It appeared that the marked fluctuations in fruit production, especially in the *Stone* and *Beefsteak* tomatoes, were correlated with high minimal (night) temperatures (at least 15°C.) occurring one month before ripening (this coincides with the period of fruit set). In the *Earliana* tomato, which sets at lower night temperatures in the greenhouse, no such correlation with periods of high minimal temperature was found. It turned out that only under optimal growing conditions was the period of time between planting and harvesting "typical," and that for the earlier plantings, which produced more tomatoes, the time necessary for fruit ripening was considerably longer. The data were analyzed in various other ways, according to fruit weight, early and late harvests, and taste.

KERCKHOFF LABORATORIES OF THE BIOLOGICAL SCIENCES,
CALIFORNIA INSTITUTE OF TECHNOLOGY,
PASADENA 4, CALIFORNIA

LITERATURE CITED

- BOSWELL, V. R., O. H. PEARSON, P. WORK, H. D. BROWN, J. H. MACGILLIVRAY, H. L. SEATON, G. E. STARR, J. J. BAYLES, W. H. FRIEND, L. R. HAWTHORN, AND H. F. MORRIS. 1933. Descriptions of types of principal American varieties of tomatoes. U. S. D. A. Misc. Pub. 160: 1-23.
- PORTER, D. R., AND J. H. MACGILLIVRAY. 1942. The production of tomatoes in California. California Agric. Ext. Serv. Circ. 104: 1-63.
- THOMPSON, H. C. 1939. Vegetable crops. New York, 3rd ed., 1939: 1-578.
- TRACY, W. 1907. Tomato culture. New York, 1907.
- WENT, F. W. 1944a. Plant growth under controlled conditions. II. Thermoperiodicity in growth and fruiting of the tomato. Amer. Jour. Bot. 31: 135-150.
- . 1944b. Plant growth under controlled conditions. III. Correlation between various physiological processes and growth in the tomato plant. Amer. Jour. Bot. 31: 597-618.
- . 1945a. Simulation of photoperiodicity by thermoperiodicity. Science 101: 97-98.
- . 1945b. Plant growth under controlled conditions. V. The relation between age, light, variety and thermoperiodicity of tomatoes. Amer. Jour. Bot. 32: 469-479.
- WORK, P. 1942. The tomato. New York, 1942: 1-135.

FLOWERING OF PERUVIAN CUBE, *LONCHOCARPUS UTILIS* A. C. SMITH, INDUCED BY GIRDLING¹

William C. Cooper, Albert L. Burkett, and Alejandro Herr

THE CULTIVATION of cube or barbasco, a native rotenone-bearing plant of South America, now occupies a prominent place in the agriculture of Peru. An estimated seven thousand hectares, or about 17,300 acres, of cube are now planted in the mountains of Peru. Although several species of cube occur in Peru, Krukoff and Smith (1937) considered that most of the rotenone-yielding cube in Peru is *Lonchocarpus utilis* A. C. Smith. The identity of this plant is still in doubt. Killip and Smith considered the common Peruvian "cube" conspecific with the fish poison of the Guianas, *Lonchocarpus nicou*. Macbride (1943) agreed with this opinion but transferred *L. nicou*, as well as all other Peruvian species of *Lonchocarpus* to *Derris*, a genus predominantly Old World. Yet, curiously, up to the time of this investigation not a single instance of flowering of either wild or cultivated *L. utilis* had been reported, though Killip and Smith (1930) and other workers have searched for flowering cube plants at various seasons of the year. The wild plantings were apparently all propagated from cuttings by the Indians who have been growing cube as a fish poison for centuries.

The investigation to induce flowering of cube experimentally was initiated with the aim of providing the taxonomist and geneticist with flowering plants for phylogenetic and breeding studies. Girdling the main stem of the plant was the only treatment tried that was effective in inducing flower formation in the cube plant. This present paper reports results of the first experiment.

EXPERIMENTAL RESULTS.—The plants used in this investigation were 2½ years old from cuttings and were located in the 10-hectare (25-acre) cube plantation of the Estacion Experimental Agricola de Tingo Maria, Peru. They were six to eight feet tall, were in active growth, and had produced vines at the apex which had intermingled among the various plants, producing a jungle-like growth (fig. 1).

The individual plants contained from one to three separate main branches coming from the original mother cutting, which was buried several inches below the surface of the soil. One or more branches were girdled on each of twelve plants on May 18, 1944. Girdling consisted in removing a ring of bark about one-half inch wide from the main stem of the plant near the ground. In addition to girdling of

¹ Received for publication July 30, 1945.

These investigations were made possible by funds provided through the United States Inter-Departmental Committee on Cultural and Scientific Cooperation and funds from the Government of Peru.

These investigations were conducted at the Estacion Experimental Agricola de Tingo Maria, operated through collaboration of the United States Department of Agriculture and the Ministry of Agriculture of Peru at Tingo Maria, Peru.

main stems, six small lateral branches near the top of the plant were ringed on two plants that had not had the main stems girdled.

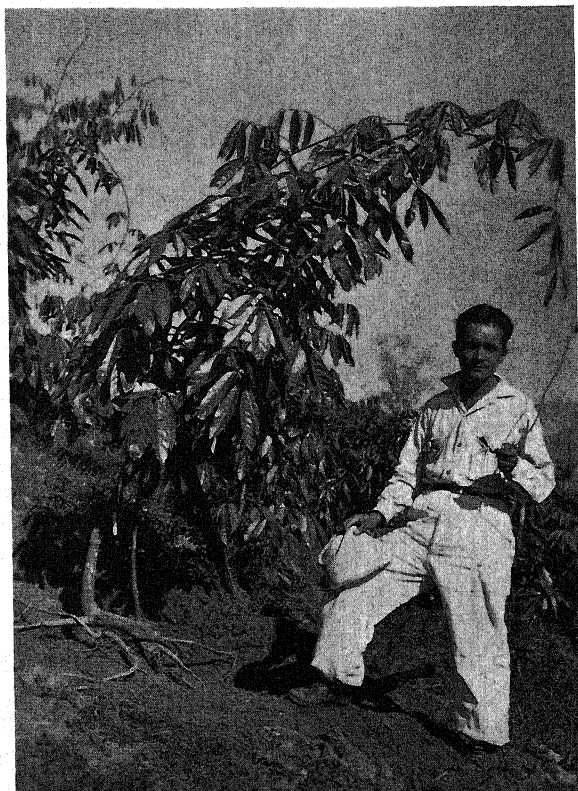


Fig. 1. Cube plant 2½ years old from the Tingo Maria cube plantation. The soil has been removed to expose the larger roots.

During the next three months vegetative growth was slowed down considerably on the girdled plants. Callus tissue formed on all of the ringed areas, and nearly all the plants showed a marked swelling of the stem just above the girdled area. No flower buds were observed as late as August 22, 1944.

The first flowers (fig. 2) were noted on September 10, 1944, approximately four months from the time of girdling. During the week following September 10, all of the girdled branches flowered profusely on that portion of the branch which was above the girdle. Only the girdled stems flowered on plants that also had several untreated main stems. On plants that had only secondary branches girdled, only the girdled branches flowered. No fruit was set on any of the girdled flowering branches.

At the time of the flowering of the girdled plants none of the neighboring ungirdled plants produced flowers. After a thorough search of each of the 5,000



Fig. 2. Flowers of cube occurring on a girdled branch. Approximately $\frac{2}{3}$ natural size.

plants in the station cube plantation, however, five ungirdled plants were observed producing flowers. The flower spikes on these plants were much smaller than those produced on the girdled plants.

In addition to the girdling in May, 25 other cube plants were girdled in July, 1944. These plants did not flower in September, 1944, which was the time that the plants girdled in May flowered.

DISCUSSION.—These preliminary results apparently indicate that under some conditions girdling will induce flowering in cube. From the present data we do not know whether girdling in May is definitely necessary in order to induce flowers. We know only that girdling in May is effective in inducing flowers in September, while girdling in July is not. Further experiments in which plants are girdled at various seasons of the year are required to determine the most effective season for girdling.

The natural flowering found at the time of the flowering of the girdled cube plants may be accounted for by accidental injury to the stem or root of the plants, which might give an effect similar to that of girdling. The small percentage of natural flowering and the possibility that, even when girdled, flowering may take place only in mid-september, could account for the failure of earlier workers to find cube flowers.

Because of their taxonomic value, herbarium materials bearing flowers induced by this experimentation are deposited in the Herbarium of the National Arboretum, Washington, D. C.

Just what happens in cube after girdling which results in flower formation is, of course, a matter of conjecture. The literature abounds in instances where girdling has influenced flowering and fruitset of various plants. Where flowering was induced by girdling, however, it was always a matter of inducing earlier or more flowering than normal. In the case of cube, except on rare occasions, only girdled plants flowered.

SUMMARY

Girdling the main branches of cube plants in Peru in May has been found effective in inducing flowering in September. This is a practical field method with cube which should prove a great aid to both the genetical and taxonomic studies with cube.

U. S. DEPARTMENT OF AGRICULTURE,
ORLANDO, FLORIDA, AND
ESTACION EXPERIMENTAL AGRICOLA DE TINGO MARIA,
TINGO MARIA, PERU

LITERATURE CITED

- KILLIP, E. P., AND A. C. SMITH. 1930. The identity of South American fish poisons, "cube" and "timbo." Jour. Washington (D.C.) Acad. Sci. 20: 74-81.
KRUKOFF, B. A., AND A. C. SMITH. 1937. Rotenone-yielding plants of South America. Amer. Jour. Bot. 24: 573-586.
MACBRIDE, J. F., 1943. Flora of Peru, Field Mus. Publ. Bot. 13, pt. 3: 263.

THE SPECIES CONCEPT IN FUSARIUM WITH REFERENCE TO DISCOLOR AND OTHER SECTIONS¹

William C. Snyder and H. N. Hansen

A PARTIAL revision of the genus *Fusarium*, involving a reduction in the number of species, was proposed in two preceding papers (Snyder and Hansen, 1940, 1941). In the present paper the remainder of the genus is revised in keeping with the species concept adhered to in the previous articles.

In the treatments of sections *Elegans* and *Martiella*, it was the writers' conviction that the former classification was not sufficiently usable for the average worker, principally because of the difficulty in distinguishing with certainty between the large number of species involved. Consequently, in revisions based upon analytical studies of the variability of these fungi, employing the single spore method of Hansen and Smith (1932), all former species in section *Elegans* were reduced to synonymy with *F. oxysporum* (Schl.) Snyder et Hansen, and likewise the species in section *Martiella* were combined into the one species, *F. solani* (Mart.) Snyder et Hansen. A trinomial system of nomenclature was used to distinguish the plant pathogens of each of these emended species, wherein a *forma*

name was attached to that of the species to indicate relative specificity for host as well as pathogenicity.

In completing the revision of the taxonomy and nomenclature of *Fusarium*, the same single spore method of analysis was employed to determine ranges of variability, as reported upon in the earlier papers. Also, as before, judgments concerning species limits were made from the standpoint of their practicability for those working in biological sciences.

Although authentic representatives of *Fusarium* species were obtained directly or indirectly through the kindness of Dr. H. W. Wollenweber for the sections *Arachnites*, *Sporotrichiella*, *Roseum*, *Arthrosporiella*, *Gibbosum*, *Discolor*, *Lateritium*, and *Liseola*, the bulk of the members studied were recent isolates made in this country. Isolates were received also from Dr. O. A. Reinking and Miss Helen Johann, members of the *Fusarium* Conference at Madison in 1924 (Wollenweber *et al.*, 1925). Many of the above sections were donated by Dr. John W. Oswald, and miscellaneous *Fusaria*, sent in for identification, have been received from various parts

¹ Received for publication July 23, 1945.

of the United States. Several members of sections *Eupionnotes* and *Macroconia* were isolated in California, and additional specimens were received from Dr. John Ehrlich and Dr. E. S. Luttrell from Idaho and Georgia, respectively. Cultures representative of section *Ventricosum* were also isolated in California. Of the sixteen sections into which Wollenweber and Reinking (1935) divide the genus *Fusarium*, only three small sections comprising a total of four species were not studied. These sections are *Submicrocera* and *Pseudomicrocera*, species of which to some extent are associated with insects, and *Spicarioides* containing the one species *F. decem-cellulare* Brick, a saprophyte.

The work on the sections treated in this paper started with 142 isolates, but numerous others were added as they were acquired. When single spore cultures were made, usually one to several variants eventually were obtained from each isolate, and in turn some of these variants continued to yield additional variants. Records of the microscopic and macroscopic characters of these isolates, and of their progenies over a period of three years, have provided the experimental data upon which revisions in classification were made.

The results from these studies indicate, as was the case in sections *Elegans* and *Martiella*, that the recorded number of species far exceeds that which may be distinguished readily by ordinary means, and that the criteria upon which the sections themselves are based are in many cases unreliable. Accordingly, and for reasons given later, a reduction in number of species has been made and all sections, which as a result become unnecessary, are dropped.

SECTIONS ROSEUM, ARTHROSPORIELLA, GIBBOSUM AND DISCOLOR.—All species, varieties and forms of these four sections are reduced here to one species, the name of which on the basis of priority, and usage, becomes *F. roseum* (Lk.), the description of which is emended accordingly. Those members of these sections which cause seedling blight, root or foot rot, or head blight of cereals are further distinguished by the *forma* name, *cerealis* (Cke.) to indicate pathogenesis. These pathogens then become known collectively as *F. roseum* f. *cerealis* (Cke.) n. comb., while members of this species which are not pathogenic to cereals are simply *F. roseum*.

There are several reasons which the writers believe justify this change, first of which is the undependability of certain of the section characters. Wollenweber and Reinking (1935) have separated these sections principally as follows:

Terminal chlamydospores lacking	
Intercalary chlamydospores lacking	<i>Roseum</i>
Intercalary chlamydospores present	
Sporodochia lacking	<i>Arthrosporiella</i>
Sporodochia present	<i>Gibbosum</i>
Intercalary and sometimes terminal	
Chlamydospores present	<i>Discolor</i>

It is true that other characters also supplement these, including foot cell development, degree of

curvature of the macroconidia, and color of stromata and of sclerotia if present. However, the emphasized characters concern the chlamydospores. It is evident that at best the nature of this key does not provide a clear basis for separating sections. Moreover, single spore cultures of fungi in section *Roseum* were found at times to produce variants which formed chlamydospores as abundantly as those in section *Gibbosum*, and variants of isolates in *Gibbosum* sometimes failed to produce chlamydospores. This observation has been recorded also by Oswald (1942). Representatives of section *Discolor* have been described as having at least intercalary and sometimes terminal chlamydospores, but in several isolates of *F. graminearum* Schwabe of this section, no chlamydospores at all were formed. Later, certain variants of this species were obtained which produced both types of chlamydospores.

Experience has shown that the presence or absence of sclerotia, and their color, are unstable and undependable taxonomic characters, and this has been so reported by Raillo (1935).

Color of mycelium and of stromata cannot be used to separate these sections for one of the characters common to each of them is the production of rosy colored mycelium in some part of the colony, although this is perhaps less often true in *Gibbosum* than in the other sections. This leaves only the conidial shape, the amount of foot cell development, and wall thickness as means to separate sections, all of which are intangible and extremely difficult to use, even if they were constant. The manner of conidium production, whether in sporodochia or not, is known to be an unreliable character as has been shown by Raillo (1935), Snyder and Hansen (1940 and 1941), and Hansen and Snyder (1943).

In attempting to identify a *Fusarium* which belongs to one of these sections, therefore, one often finds he is uncertain of the section to which his organism belongs. He is then confronted with the numerous descriptions of species, varieties and forms found in the four sections. The principal criteria in species separation have been size and shape of conidia, septation frequency, and color production. But these are unsuited for characterizing the closely drawn but narrowly delimited species of Wollenweber and Reinking (1935) for the reason already dealt with by the writers in preceding papers (1940, 1941) on the genus.

It is not the intent here to imply that the characters previously used for separating *Fusaria* are of no value. Nor is it to be inferred at all that no *Fusaria* can be identified, definitely, by the existing system. On the contrary, it is frequently possible to place with precision a given isolate in its proper position in Wollenweber and Reinking's (1935) classification, at a given time. But it is when an isolate seems to straddle species lines, or even section lines, that the system breaks down, for too often an isolate appears to fit possibly into a number of species but not certainly into any one. Another serious breakdown in the system occurs when

fungi which originally may have been specifically identified are re-examined and re-identified after a period in culture, for then it may be found that the first and second identifications do not agree, perhaps not even in respect to section characters.

For example, a single spore culture which agreed well with the description of *F. culmorum* (W. G. Sm.) Sacc. of section *Discolor* repeatedly produced variants which fell into the species *F. sambucinum* Fkl. of this section. Again, *F. culmorum* threw variants identified as *F. heterosporum* Nees, and as *F. flocciferum* Cda., all of section *Discolor*, a finding reported also by Oswald (1942). A single ascospore culture of *F. graminearum* (*Gibberella saubinetii* (Mont.) Sacc.) of section *Discolor* gave variants some of which closely resembled *F. avenaceum* (Fr.) Sacc. of section *Roseum*, and others which approached *F. culmorum* of section *Discolor*. Under certain conditions of growth, a culture first identified as *F. graminearum* was later placed in *F. sublunatum* Rg., and at still another time, in *F. macroceras* Wr. et Rg., all in section *Discolor*. Yet its isolation history, and pathogenicity tests showed it to be the organism known as *F. graminearum*. A similar type of apparent fluctuation in species identity occurred in members of sections *Roseum* and *Gibbosum*. A fungus first identified as *F. avenaceum* (section *Roseum*) later was placed as a variety of *F. scirpi* L. et F. in *Gibbosum*. Members of *Arthrosporiella* (non-sporodochia formers) largely seemed to be variants from sporodochia-producing members of *Roseum*, *Discolor* and *Gibbosum*.

These findings of apparent transmutations of species in the above groups of *Fusaria* are not new. Mitter (1928) reported that the specific characters for *Discolor* species were not distinctive since sal-tants derived from a given parent may show a greater divergence in critical characters than is found between species. He also concluded that the criteria used for separating sections were "entirely unsatisfactory." In other studies in these same sections of *Fusarium*, undertaken from both the cultural and pathogenicity standpoints, Oswald (1942) clearly demonstrated the unreliability of sectional as well as of species criteria in the existing classification system. He came to the conclusion that the cereal pathogens of these sections (*Discolor*, *Roseum*, *Gibbosum*) could not be distinguished with dependability. At first thought, a merging of species of these sections may seem to be on too inclusive a scale, but actually plant pathologists have been organizing subject matter on certain *Fusarium* diseases somewhat along this line for some time.

In principle, Dickson's (1939) thorough treatment of the *Fusarium* root rots and scab of cereals would seem to be in accord with this view. Under the heading of "Fusarium blight, scab of barley" Dickson cites as the "causal organism," *F. graminearum* (*Gibberella saubinetii*), *F. culmorum*, and *F. avenaceum*. The same treatment is given for wheat scab. Although several species of *Fusarium* are credited with causing this disease, they are

sometimes grouped as one. It may be true that two of these species are not known to produce ascus stages, but then not all individuals of *F. graminearum* produce an ascus stage. It is well known that sexually non-fertile individuals arise in ascomycetes (Hansen and Snyder, 1943) yet may still be members of the same imperfect species. Bennett (1935) states concerning the identification of *F. graminearum* and *F. culmorum*: "For the two fungi in question, the distinctive features are that *Gibberella saubinetii* (*F. graminearum*) produces perithecia but no chlamydospores, whilst *F. culmorum* produces chlamydospores but no perithecia." But the present writers and also Oswald (1942) have obtained abundant chlamydospores in certain cultures of *F. graminearum*. Does, then, the capacity to produce perithecia alone constitute an acceptable basis for the separation of *F. culmorum* from *F. graminearum*? If so, then what is to be done with the variants of *F. graminearum* which can no longer produce perithecia, or those which for some reason do not complete this stage?

Bennett (1935), Oswald (1942), Sprague (1944) and others have shown that several species in sections *Discolor*, *Roseum* and *Gibbosum* are associated in nature with root rot of cereals, and that by means of pathogenicity tests these species are in some degree pathogenic. They have shown that the type of symptoms produced by these species is the same, whether infection be on the below-ground parts or on the heads of cereals. Bennett cites not only *F. graminearum*, *F. culmorum* (both of section *Discolor*) and *F. avenaceum* (section *Roseum*) as the cause of cereal root rot but also *F. equiseti* (Cda.) Sacc. (section *Gibbosum*) and *F. sambucinum* (section *Discolor*) (weakly pathogenic). Gordon and Sprague (1941) have found that a variety of *F. scirpi* (section *Gibbosum*) is also pathogenic on cereals. Oswald added still other species to this list of pathogens, namely, *F. flocciferum*, *F. reticulatum* Mont., and *F. sublunatum*, of section *Discolor*, at the same time pointing out that the more intensively these species and their variants are studied in culture, the longer the list of fungi may become. Wollenweber and Reinking (1935) themselves mention *F. heterosporum*, *F. scirpi*, *F. equiseti*, *F. arthrosporioides* Sherb., and other members of these four sections as being closely associated with root rots of grain in addition to several other species.

Oswald has found that the variants of *F. graminearum* derived by single-spore culture and those of *F. culmorum*, such as *F. sublunatum* and *F. sambucinum* respectively, are less pathogenic than the parent types. Isolates of these fungi direct from nature also show differences in virulence. In fact some isolates of *F. sambucinum* from non-cereal hosts for example, have been found (Oswald, 1942) non-pathogenic on cereals. Here is another point of difficulty in the existing classification, for how is one to distinguish between the pathogenic and non-

pathogenic forms of *F. sambucinum* or, similarly, the parasites and saprophytes in other species?

There are diseases other than those of cereals the cause of which has been ascribed to a group of several *Fusarium* species of the sections now condensed into *F. roseum*. Wollenweber and Reinking (1935), for example, list *F. culmorum*, *F. avenaceum*, *F. semitectum*, *F. sambucinum*, *F. equiseti* and *F. scirpi*, among others, as the cause of tomato fruit rot. All of these binomials are synonyms of our *F. roseum*. Wiant (1937) in investigations on the market diseases of melons found eleven different species or varieties of *Fusarium* associated with fruit decay, and all of these except two, which were represented by single isolates, belong to sections *Roseum*, *Arthrosporiella*, *Gibbosum*, and *Discolor*. The morphologic similarity of the members of these sections is paralleled by their biologic resemblances, facts which seem to require their treatment in one rather than in several groups.

It is in part due to such observations that the writers propose the combining of sections *Roseum*, *Arthrosporiella*, *Gibbosum* and *Discolor* into the one morphologic species, *F. roseum*, and of the pathogenic forms of this species which attack cereals under the trinomial, *F. roseum* f. *cerealis*. The name *F. roseum* is one of the oldest binomials in the genus *Fusarium*, having been applied in 1809 by Link (1809), the year the genus was established. This species later became split into a number of species. Wollenweber and Reinking (1935) in their synonymy refer to *F. roseum* and varieties as being in part *F. graminearum*, *F. sambucinum*, *F. graminum* Cda., *F. semitectum* Berk. et Rav., *F. avenaceum*, *F. culmorum*, *F. scirpi*, or their varieties. These are species in sections *Discolor*, *Gibbosum*, *Arthrosporiella* and *Roseum*, and are among the species which the writers propose to recombine under the original binomial *F. roseum* as conceived by Link well over a century ago.

Certain of the *Fusaria* grouped here under *F. roseum* have been reported to cause diseases other than root rot and scab of cereals. For example, root rots of alfalfa, broad bean, and carnation, and a wilt of potato have been attributed to *F. avenaceum*. Also, *F. culmorum* has been identified with a disease on carnation. Should later work demonstrate that the pathogens of these or other diseases caused by representatives of *F. roseum* as now constituted are physiologically specialized as to host and distinct from *F. roseum* f. *cerealis*, then new *formae* should be established for them.

The perithecial stages of the species in the four sections considered here, in so far as they have been described heretofore, fall into six species (with varieties) of *Gibberella*. These species have been distinguished from each other by slight differences in perithecial and spore measurements. In keeping with the reduction of the imperfect species under one name, the perfect species names are likewise reduced to one, namely, *Gibberella roseum* n. comb. This binomial is selected rather than others here-

tofore used, such as *G. saubinetii*, since *F. roseum* was described prior to the discovery of any *Gibberella* stage of a *Fusarium*. It seems natural, therefore, that the species name *roseum* should have priority in referring to any individual or individuals of *F. roseum* in which the perfect stage occurs or later may be found to occur. *Gibberella roseum* f. *cerealis* n. comb. becomes the name of the perithecial stage of *F. roseum* f. *cerealis*, and the synonym of *Gibberella saubinetii*.

SECTION LATERITIUM.—Although the species of this section show distinct relationship to the species *F. roseum*, and Wollenweber and Reinking (1935) give Link's *F. roseum*, in part, as a synonym of *F. lateritium* Nees, the writers feel that there is some practical advantage in maintaining for the present a separate species to represent this section. Accordingly, in revising the section *Lateritium* on bases of the same nature as those discussed for *F. roseum*, all species and varieties of the section are placed in the one species, *F. lateritium*. This binomial is selected on the basis of priority, the species having been described by Nees in 1817. Since no highly parasitic members of this group have been recognized heretofore, no *formae* of the emended species, *F. lateritium*, are created at this time.

The ascigerous stages recorded for section *Lateritium* are combined under the one species name, *Gibberella lateritium* (Nees) n. comb., in view of their close similarity.

SECTION LISEOLA.—This section, which includes other *Fusaria* with a *Gibberella* stage, is well known to plant pathologists through *F. moniliforme* Sheld. and its varieties. The section as formerly constituted, also contained two other species, *F. lactis* Pir. et Rib. and *F. neoceras* Wr. et Rg., with their varieties.

The principal character used to divide members of this section into two groups is whether the microconidia are borne in chains or false heads. Further subdivision has been based on the manner in which the macroconidia are produced, whether by sporodochia, pionnotes, or neither; and on spore size. These criteria, as used, have been shown by the writers to be unsatisfactory for separating the narrowly defined species of Wollenweber and Reinking in the case of other sections (Snyder and Hansen, 1940, 1941), and the same applies here. Furthermore, single spore cultures of *F. moniliforme* have been observed which, undisturbed, clearly bear the microconidia simultaneously both in chains and in false heads. This character (microconidia borne in chains) is, therefore, an unstable feature of the section which has some usefulness when present, but can not be used reliably to separate even varieties. Also, it has already been shown clearly that the presence or absence of sporodochia or pionnotes is of no value whatever in distinguishing species, any more than are slight differences in size or septation frequency. The species and varieties of *Liseola*, therefore, are reduced to one species, *F.*

moniliforme (Sheld.) the name of which is selected on the basis of usage as being that best known and best established in the section.

For similar reasons, *Gibberella moniliforme* (Wineland) becomes the name of the perfect stage of this species. The binomial *F. moniliforme*, was in use prior to the discovery of the perfect stage, and therefore its continued use is most appropriate where the perfect stage of any member of the species is involved.

Many papers have been published dealing with parasitism in the section *Liseola*. Seedling, stalk, and ear rots of corn and of other cereals have been identified with *F. moniliforme*, or a variety of it, in this country for many years. Much has been written of the bakanae disease of rice in the Orient, and of the pokkah-boeng disease of sugar cane in those areas in which the crop is grown extensively. Both of these diseases have been attributed to *F. moniliforme* or *F. moniliforme* v. *subglutinans* Wr. et Rg. One or both of these names have been applied to fungi reported to cause diseases also of various other crops, including fig, cotton and banana. Since there is not yet conclusive evidence that these various diseases are caused by physiological forms, specialized as to hosts, no attempt is made at this time to establish *formae* within the emended species *F. moniliforme*.

SECTION SPOROTRICHIELLA.—Separated by the presence or absence of sporodochia and pionnotes, spore septation and size, the species and varieties of this section closely approach *F. roseum* except for odd-shaped microconidia. It is a question as to how much taxonomic emphasis should be placed on the shape of the microconidia, for it is not an altogether dependable character. Bennett (1935) reports that the pyriform microconidia of *F. tricinatum* (Cda.) Sacc. may disappear with age, and also, that such microconidia may be found in *F. sambucinum*. Gordon (1944) has reported the frequent association of *F. poae* Peck with cereal seed in Canada. This species also has been identified both with a bud rot of carnation and a disease of certain grasses. However, *F. avenaceum*, *F. reticulatum* and *F. tricinatum*, etc., have also been reported to produce the same disease on these hosts. The members of the section clearly are variants of the same fungus, and are here so designated under the older species name, *F. tricinatum*, while the pathogen on carnation is indicated by the trinomial *F. tricinatum* f. *poae* (Pk.) n. comb.

SECTION ARACHNITES.—Members of this section have been separated principally on the characters of septation frequency, substrate, and spore size. Variability in isolates of *F. nivale* (Fr.) Ces. has been found great enough to embrace the other species and varieties of the section. Consequently, the species *F. nivale* is emended in agreement with the section description, and *Calonectria nivale* (Fr.), used to designate the perfect stage of this species in so far as it occurs. Furthermore, the cereal pathogen of the emended species is given a forma name,

F. nivale f. *graminicola* (Berk. et Brme) n. comb., with a perfect stage of *Calonectria nivale* f. *graminicola* (Berk. et Brme) n. comb. The morphology and role of *F. nivale* as a cereal pathogen brings this fungus, like *F. tricinatum*, also into close relationship with *F. roseum*.

SECTIONS EUPIONNOTES AND MACROCONIA.—These sections contain nine species and varieties, mostly unimportant from the standpoint of plant pathology, but not infrequently encountered. The perfect stages in the genus *Nectria* have been collected for the work reported here from dead bark of various trees and shrubs, from stromata of Sphaeriaceous fungi, from blister rust lesions, scale insects, and from decaying vegetable matter including pumpkin rind. Some of these fungi when first recovered from nature seemed to fall with some uncertainty in the species *F. aqueductum* (R. et R.) Lagh., *F. sphaeria* Fkl., *F. cavispermum* Cda., *F. merismoides* Cda., *F. dimerum* Penzig, and *F. flavum* (Fr.) Wr. In gross cultural characters they resemble one another in that the colonies are mostly slow-growing with scant mycelium, consisting largely of conidial slime, usually bright colored. Later, variants were obtained which consisted of tufted mycelial colonies which produced less conidial slime but which were still slow-growing. Although no perithecial stage of *F. dimerum* is mentioned by Wollenweber and Reinking, the writers obtained a typical *F. dimerum* from several single ascospore cultures made from *Nectria* perithecia found on a rotted pumpkin fruit. Later, these same cultures produced longer macroconidia, like those of *F. merismoides*. Likewise, *F. flavum* yielded at times larger macroconidia.

Not only have the small-spored species, *F. dimerum*, and *F. flavum*, of Wollenweber and Reinking been found to produce in culture much larger macroconidia, like those of *F. merismoides* and *F. expansum* Schl., but also, single spores from nature which approached the size of those described for *F. gigas* Speg. yielded in culture smaller conidia of the kind described for *F. coccophilum* Wr. and *F. expansum*. These findings support the view that the difference in size, shape, and septation, used as bases for separating species in these two sections, is to a certain extent merely the reflection of environmental influences, and to a certain extent represents variants of the same fungus.

It has been the experience of the writers, in working with the members of these two sections, that spore shapes so merge one into another, and that spore sizes are so extremely variable and clearly determined in large part by the environment (including substrate) as to make impractical the delimitation of species as heretofore attempted. Because of the impracticability of separating these species the writers judge it advisable to unite the members of the two sections into one species, *F. episphaeria* (Tode) n. comb. This name is taken from the ascomycetous species, *Nectria episphaeria* (Tode) Fr., described in 1791, to which it belongs.

Likewise, the variability in size and even shape of the perithecia as well as in ascospore sizes of the perfect species of these sections seem to make untenable the number of species distinguished. These are placed here into one species, *Nectria episphaeria* (Tode), into which the perfect stages of members of *F. episphaeria* fall (where and if found).

Since there is evidence that one of the constituents of the present species, *F. episphaeria*, parasitizes scale insects, the form *F. episphaeria* f. *coccophila* (Desm.) n. comb. is established for it with an ascigerous stage in *Nectria episphaeria* f. *coccophila* (Desm.) n. comb.

SECTION SPICARIOIDES.—Only one species has been described for this section (*F. decemcellulare*). This fungus was not available for study, and is retained for the time being as a doubtful species under the name *F. rigidiusculum* (Berk. et Brme.) n. comb., which name is taken from its earlier described perfect stage, *Calonectria rigidiuscula* (Berk. et Brme.) Sacc.

SECTIONS SUBMICROCERA AND PSEUDOMICROCERA.—Members of these sections were not studied. However, because of the similarity in the descriptions of the species of these sections they are here arbitrarily combined into one, which on the basis of priority is called *F. ciliatum* (Lk.). The various binomials applied to the ascus stages are also reduced to one, namely, *Calonectria ciliatum* n. comb. This species is listed here as a doubtful species also.

SECTION VENTRICOSUM.—Since publication of the revision of section *Martiella*, isolates of the one species (*F. argillaceum*) (Fr.) Sacc. of section *Ventricosum* have been obtained and studied. As a result, *F. argillaceum* is found to be a synonym of *F. solani*, and the section characters are merged into the description of the latter species, with a ascus stage, *Hypomyces solani* (Rke. et Berth.) Snyder et Hansen.

REVISION AND SYNONYMY OF SECTIONS OTHER THAN ELEGANS AND MARTIELLA.—Names of the species retained in the following classification are the same as those previously used, but the descriptions have been changed and broadened so as to include the species now listed as synonyms. The synonyms given include those representing first descriptions of the fungi in question. Also listed are the synonyms of Wollenweber and Reinking's species, thus relating the new nomenclature used here directly to that in their "Die Fusarien" (1935).

Fusarium episphaeria (Tode) n. comb. Species characters and limits equivalent to the combined characterizations of *Fusarium* species in sections *Eupionnotes* and *Macroconia*, as used by Wollenweber and Reinking (1935).

Syn. *Fusarium aquaeductuum* (Radlk. et Rabh. pr. p.) Lagh. Wr. and Rg. (1935).

F. aquaeductuum (Radlk. et Rabh. pr. p.) Lagh. v. *medium* Wr. Wr. and Rg. (1935).

F. buxicola Sacc. Wr. and Rg. (1935).

F. cavispermum Cda. Wr. and Rg. (1935).

F. dimerum Penz. Wr. and Rg. (1935).

F. dimerum Penz. v. *nectrioides* Wr. Wr. and Rg. (1935).

F. dimerum Penz. v. *pusillum* Wr. Wr. and Rg. (1935).

F. dimerum Penz. v. *violaceum* Wr. Wr. and Rg. (1935).

F. expansum Schl. Wr. and Rg. (1935).

F. flavum (Fr.) Wr. Wr. and Rg. (1935).

F. gigas Speg. Wr. and Rg. (1935).

F. melanochlorum (Casp.) Sacc. Wr. and Rg. (1935).

F. merismoides Cda. Wr. and Rg. (1935).

F. merismoides Cda. v. *chlamydosporale* Wr. Wr. and Rg. (1935).

F. merismoides Cda. v. *crassum* Wr. Wr. and Rg. (1935).

F. sphaeria Fkl. Wr. and Rg. (1935).

FUSARIUM episphaeria (Tode) Snyder et Hansen f. *coccophila* (Desm.) n. comb.

Syn. *Microcera coccophila* Desm.

Desmazieres (1848).

Fusarium coccophilum (Desm.) Wr. et Rg. Wr. and Rg. (1935).

Nectria episphaeria (Tode) emend. Characterization equivalent to that of the combined species of *Nectria* in sections *Eupionnotes* and *Macroconia*, as used by Wollenweber and Reinking (1935).

Syn. *Sphaeria episphaeria* Tode. Tode (1791).

Nectria desmazierii Becc. et DNtrs. Wr. and Rg. (1935).

N. episphaeria (Tode) Fr. Wr. and Rg. (1935).

N. episphaeria (Tode) Fr. v. *coronata* Wr. Wr. and Rg. (1935).

N. flavo-viridis (Fkl.) Wr. Wr. and Rg. (1935).

N. leptosphaeriae Niessl. Wr. and Rg. (1935).

N. stilbosporae Tul. Wr. and Rg. (1935).

NECTRIA episphaeria (Tode) Snyder et Hansen f. *coccophila* (Desm.) n. comb.

Syn. *N. coccophila* (Tul.) Wr. et Rg. Wr. and Rg. (1935).

Sphaerostilbe aurantiicola (B. et Br.) Petch. Petch (1922).

Fusarium nivale (Fr.) emend. Characterization the same as that of the combined species of *Fusarium* in section *Arachnites*, as used by Wollenweber and Reinking (1935).

Syn. *Lanosa nivalis* Fr. pr. p. Fries (1825).

Fusarium kühni (Fkl.) Sacc. Wr. and Rg. (1935).

F. larvarum Fkl. Wr. and Rg. (1935).

F. nivale (Fr.) Ces. pr. p. Wr. and Rg. (1935).

F. nivale (Fr.) Ces. v. *majus* Wr. pr. p. Wr. and Rg. (1935).

FUSARIUM nivale (Fr.) Snyder et Hansen f. *graminicola* (Berk. et Brme.) n. comb.

Syn. *F. nivale* (Fr.) Ces. pr. p. Wr. and Rg. (1935).

F. nivale (Fr.) Ces. v. *Majus* Wr. pr. p. Wr. and Rg. (1935).

Calonectria nivale (Fr.) emend. Characterization the same as that of the combined species of *Calonectria* in section *Arachnites*, as given by Wollenweber and Reinking (1935).

Syn. *Calonectria graminicola* (Berk. et Brme.) Wr. pr. p. Wr. and Rg. (1935).

C. graminicola (Berk. et Brme.) Wr. v. *neglecta* Krampe pr. p. Wr. and Rg. (1935).

CALONECTRIA nivale (Fr.) Snyder et Hansen f. **graminicola** (Berk. et Brme.) n. comb.

Syn. *Nectria graminicola* (Berk. et Brme.) Berk. and Brme. (1859).

Calonectria graminicola (Berk. et Brme.) Wr. pr. p. Wr. and Rg. (1935).

C. graminicola (Berk. et Brme.) Wr. v. *neglecta* Krampe pr. p. Wr. and Rg. (1935).

Fusarium tricinctum (Cda.) emend. Characterization is the same as that of the combined *Fusarium* species in section *Sporotrichiella*, as given by Wollenweber and Reinking (1935).

Syn. *Selenosporium tricinctum* Sda. Cda. (1837).
F. chlamydosporum Wr. et Rg. Wr. and Rg. (1935).

F. poae (Pk.) Wr. pr. p. Wr. and Rg. (1935).

F. tricinctum (Cda.) Sacc. Wr. and Rg. (1935).

F. sporotrichioides Sherb. pr. p. Wr. and Rg. (1935).

F. sporotrichioides Sherb. v. *Minus* Wr. Wr. and Rg. (1935).

FUSARIUM tricinctum (Cda.) Snyder et Hansen f. **poae** (Pk.) n. comb.

Syn. *Sporotrichum poae* Peck. Peck (1903).

Fusarium poae (Peck) Wr. pr. p. Wr. and Rg. (1935).

F. sporotrichioides Sherb. pr. p. Wr. and Rg. (1935).

Fusarium roseum (Lk.) emend. Species characters and limits the sum total of those given for sections *Roseum*, *Arthrosporiella*, *Gibbosum* and *Discolor*, by Wollenweber and Reinking (1935).

Syn. *F. roseum* Lk. Link (1809).

F. anguioides Sherb. Wr. and Rg. (1935).

F. arthrosporioides Sherb. Wr. and Rg. (1935).

F. avenaceum (Fr.) Sacc. pr. p. Wr. and Rg. (1935).

F. avenaceum (Fr.) Sacc. f. 1 Wr. et Rg. Wr. and Rg. (1935).

F. avenaceum (Fr.) Sacc. v. *pallens* Wr. Wr. and Rg. (1935).

F. avenaceum (Fr.) Sacc. v. *volutum* Wr. et Rg. Wr. and Rg. (1935).

F. bactridioides Wr. Wr. and Rg. (1935).

F. camptoceras Wr. et Rg. Wr. and Rg. (1935).

F. concolor Rg. Wr. and Rg. (1935).

F. culmorum (W.G.Sm.) Sacc. pr. p. Wr. and Rg. (1935).

F. culmorum (W.G.Sm.) Sacc. v. *cereale* (Cke.) Wr. pr. p. Wr. and Rg. (1935).

F. deToniaum Sacc. Wr. and Rg. (1935).

F. diversisporum Sherb. Wr. and Rg. (1935).
F. equiseti (Cda.) Sacc. pr. p. Wr. and Rg. (1935).

F. equiseti (Cda.) Sacc. v. *bullatum* (Sherb.) Wr. Wr. and Rg. (1935).

F. flocciferum Cda. pr. p. Wr. and Rg. (1935).

F. graminearum Schwabe pr. p. Wr. and Rg. (1935).

F. graminum Cda. Wr. and Rg. (1935).

F. heterosporum Nees. Wr. and Rg. (1935).

F. heterosporum Nees v. *congoense* Wr. Wr. and Rg. (1935).

F. macroceras Wr. et Rg. Wr. and Rg. (1935).

F. reticulatum Mont. pr. p. Wr. and Rg. (1935).

F. reticulatum Mont. f. 1 Wr. Wr. and Rg. (1935).

F. reticulatum Mont. v. *negundinis* (Sherb.) Wr. Wr. and Rg. (1935).

F. retusum Wellman. Wellman (1943).

F. sambucinum Fkl. pr. p. Wr. and Rg. (1935).

F. sambucinum Fkl. f. 1 Wr. Wr. and Rg. (1935).

F. sambucinum Fkl. f. 2 Wr. Wr. and Rg. (1935).

F. sambucinum Fkl. f. 4 Wr. Wr. and Rg. (1935).

F. sambucinum Fkl. f. 5 Wr. Wr. and Rg. (1935).

F. sambucinum Fkl. f. 6 Wr. Wr. and Rg. (1935).

F. sambucinum Fkl. 1. *minus* Wr. Wr. and Rg. (1935).

F. scirpi Lamb. et Fautr. pr. p. Wr. and Rg. (1935).

F. scirpi Lamb. et Fautr. v. *acuminatum* (Ell. et Ev.) Wr. pr. p. Wr. and Rg. (1935).

F. scirpi Lamb. et Fautr. v. *caudatum* Wr. Wr. and Rg. (1935).

F. scirpi Lamb. et Fautr. v. *compactum* Wr. Wr. and Rg. (1935).

F. scirpi Lamb. et Fautr. v. *filiferum* (Preuss.) Wr. Wr. and Rg. (1935).

F. scirpi Lamb. et Fautr. v. *longipes* (Wr. et Rg.) Wr. Wr. and Rg. (1935).

F. semitectum Berk. et Rav. Wr. and Rg. (1935).

F. semitectum Berk. et Rav. v. *majus* Wr. Wr. and Rg. (1935).

F. sublunatum Rg. Wr. and Rg. (1935).

F. sublunatum Rg. v. *elongatum* Rg. Wr. and Rg. (1935).

F. tumidum Sherb. Wr. and Rg. (1935).

F. tumidum Sherb. v. *humi* Rg. Wr. and Rg. (1935).

F. trichothecioides Wr. Wr. and Rg. (1935).

FUSARIUM roseum (Lk.) Snyder et Hansen f. **cerealis** (Cke.) n. comb.

Syn. *Fusisporium cerealis* Cke. Cooke (1878).

Fusarium avenaceum (Fr.) Sacc. pr. p. Wr. and Rg. (1935).

- F. culmorum* (W.G.Sm.) Sacc. pr. p. Wr. and Rg. (1935).
F. culmorum (W.G.Sm.) Sacc. v. *cereale* (Cke.) Wr. Wr. and Rg. (1935).
F. graminearum Schwabe pr. p. Wr. and Rg. (1935).
F. equiseti (Cda.) Sacc. pr. p. Bennett (1935).
F. flocciferum Cda. pr. p. Oswald (1942).
F. graminearum Schwabe pr. p. Wr. and Rg. (1935).
F. reticulatum Mont. pr. p. Oswald (1942).
F. sambucinum Fkl. pr. p. Bennett (1935).
F. scirpi Lamb. et Fautr. pr. p. Wr. and Rg. (1935).
F. scirpi Lamb. et Fautr. v. *acuminata* (Ell. et Ev.) Wr. pr. p. Gordon and Sprague (1941).
- Gibberella roseum** (Lk.) n. comb. Species characters and limits represented by combined descriptions of the *Gibberella* fungi given in sections *Gibbosum* and *Discolor* by Wollenweber and Reinking (1935).
 Syn. *G. accuminata* Wr. Wr. and Rg. (1935).
G. cyanea (Sollm.) Wr. Wr. and Rg. (1935).
G. heterochroma Wr. Wr. and Rg. (1935).
G. intricans Wr. Wr. and Rg. (1935).
G. pulicaris (Fr.) Sacc. Wr. and Rg. (1935).
G. pulicaris (Fr.) Sacc. v. *minor* Wr. Wr. and Rg. (1935).
G. saubinetii (Mont.) Sacc. pr. p. Wr. and Rg. (1935).
G. zeae (Schw.) Petch pr. p. Petch (1936).
- GIBBERELLA roseum** (Lk.) Snyder et Hansen f. **cerealis** (Cke.) n. comb.
 Syn. *G. saubinetii* (Mont.) Sacc. pr. p. Wr. and R. (1935).
G. zeae (Schw.) Petch pr. p. Petch (1936).
- Fusarium lateritium** (Nees) emend. Characterization same as that given for all species in section *Lateritium* by Wollenweber and Reinking (1935).
 Syn. *F. lateritium* Nees. Nees (1817), Wr. and Rg. (1935).
F. lateritium Nees v. *longum* Wr. Wr. and Rg. (1935).
F. lateritium Nees v. *majus* Wr. Wr. and Rg. (1935).
F. lateritium Nees v. *minus* Wr. Wr. and Rg. (1935).
F. lateritium Nees v. *mori* Desm. Wr. and Rg. (1935).
F. lateritium Nees v. *uncinatum* Wr. Wr. and Rg. (1935).
F. sarcochroum (Desm.) Sacc. Wr. and Rg. (1935).
F. stilboides Wr. Wr. and Rg. (1935).
- Gibberella lateritium** (Nees) n. comb. Species characters and limits represented by combined descriptions of *Gibberella* fungi in section *Lateritium* as given by Wollenweber and Reinking (1935).
 Syn. *G. baccata* (Wallr.) Sacc. Wr. and Rg. (1935).
G. baccata (Wallr.) Sacc. v. *major* Wr. Wr. and Rg. (1935).
G. baccata (Wallr.) Sacc. v. *moricola* (DNtrs) Wr. Wr. and Rg. (1935).
G. pseudopulicaris Wr. Wr. and Rg. (1935).
- Fusarium moniliforme** (Sheld.) emend. Characterization same as that of the combined species in section *Liseola* as used by Wollenweber and Reinking (1935).
 Syn. *F. moniliforme* Sheld. Sheldon (1904), Wr. and Rg. (1935).
F. lactis Pir. et Rib. Wr. and Rg. (1935).
F. moniliforme Sheld. v. *anthophilum* (A. Br.) Wr. Wr. and Rg. (1935).
F. moniliforme Sheld. v. *minus* Wr. Wr. and Rg. (1935).
F. moniliforme Sheld. v. *subglutinans* Wr. et Rg. Wr. and Rg. (1935).
F. neoceras Wr. et Rg. Wr. and Rg. (1935).
- Gibberella moniliforme** (Sheld.) emend. Characterization same as combined descriptions of all *Gibberella* fungi in section *Liseola* as given by Wollenweber and Reinking (1935).
 Syn. *G. fujikuroi* (Saw.) Wr. Wr. and Rg. (1935).
G. fujikuroi (Saw.) Wr. v. *subglutinans* Edw. Wr. and Rg. (1935).
G. moniliformis (Sheld.) Wineland. Wine-land (1924), Wr. and Rg. (1935).
- Doubtful Species*
- Fusarium rigidiuscula** (Brick) n. comb.
 Syn. *F. decemcellulare* Brick. Brick (1908), Wr. and Rg. (1935).
Calonectria rigidiuscula (Berk. et Brme.) Sacc. Wr. and Rg. (1935).
- Fusarium ciliatum** (Lk.) emend. Characterization is that of the combined species of *Fusarium* in sections *Microcera* and *Pseudomicrocera*, as given by Wollenweber and Reinking (1935).
 Syn. *Atracticum ciliatum* Lk. pr. p. Link (1816).
Fusarium ciliatum Lk. Wr. and Rg. (1935).
F. cerasi Roll. et Ferry. Wr. and Rg. (1935).
F. juruanum P. Henn. Wr. and Rg. (1935).
F. orthoconium Wr. Wr. and Rg. (1935).
- Calonectria ciliatum** (Lk.) n. comb. Characters represented by the combined descriptions of *Calonectria* stages in sections *Microcera* and *Pseudomicrocera*, as used by Wollenweber and Reinking (1935).
 Syn. *C. decora* (Wallr.) Sacc. Wr. and Rg. (1935).
C. diploa (Berk. et Curt.) Wr. Wr. and Rg. (1935).
- DISCUSSION.—Revision of the taxonomy and nomenclature of the genus *Fusarium* completed in this paper was undertaken primarily from the practical standpoint of applied mycology. The writers and others (largely plant pathologists) who have frequent occasion to diagnose *Fusarium* diseases long have felt the need for simplification in *Fusarium*

classification. "Die Fusarien" (of Wollenweber and Reinking, 1935) contributed immensely to the convenience of the worker by bringing together in one place, under exceptional, detailed organization, an enormous amount of scattered information. It has not, however, provided the plant pathologist with a sufficiently usable taxonomic system for the identification of the 65 species, 56 varieties and 22 forms, totaling 143 *Fusaria*, distinguished within the genus in that volume. Although retaining much of the basic organization of these fungi employed by Wollenweber and Reinking, the writers distinguish altogether only eight species, no varieties, and 34 forms (mostly in *F. oxysporum*), totaling 42 *Fusaria*. In addition, two doubtful species are listed. In this system, therefore, the investigator who wishes to identify an isolate of *Fusarium* has eight morphologically distinct unit groupings (species) to consider instead of the former 121 morphological groupings (species and varieties). The 34 forms are determined solely upon distinctive pathogenesis and not by morphological or cultural characters.

The writers are fully aware that their system presents its difficulties. There will always be questions concerning the placement of border-line species and of variants. Perhaps the principal benefit the system provides, if no other, lies in the great reduction in the number of species border-lines obtained, by broadening the species retained. Naturally this will serve to reduce the frequency with which border-line questions arise. Later work may show that the writers have been a bit conservative in their reduction of the number of species, and that a more satisfactory handling of the *Fusaria* may require that the eight species now retained be further reduced.

Inevitably questions have arisen during these studies concerning the classification of the ascus stages, insofar as they have been seen. The *Fusaria* are known to be represented in four ascomycetous genera, namely *Calonectria*, *Gibberella*, *Hypomyces* and *Nectria*. The first two genera have one to three septate ascospores and are distinguished from each other principally by perithecial color. The last two genera have 1-septate ascospores and usually the same perithecial color, but are held to differ from each other in some uncertain characteristic of the supporting stromata. The retention of both *Calonectria* and *Gibberella* on the basis of a pigment difference has been questioned before. Bennett (1933) has obtained perithecia of *F. nivale* which were blue-black in color, a character which could place them in *Gibberella* instead of in *Calonectria*. Likewise, the distinction between *Hypomyces* and *Nectria* seems uncertain. The writers believe that a study of these four genera will result in their reduction to two, differentiated perhaps on the basis of ascospore characters only, perithecial color being disregarded.

In regard to the species of these four perfect genera, it is notable that, upon the basis of mor-

phology alone, it would be difficult to distinguish with certainty, for example, between the ascigerous stages of any of the *Calonectria* or *Gibberella* species which have been described by Wollenweber and Reinking in "Die Fusarien." Certainly these facts illustrate forcibly that a fungus bearing an ascigerous stage must be known in all its conidial stages (if it produces such) in order to be properly classified, and that the presence of the ascus stage alone may not permit its identification to species in the Ascomycetes any more correctly than would the knowledge of one phase of its imperfect stage (microconidia or macroconidia, alone) permit its correct placement in the Fungi Imperfecti.

The basis for the revision in *Fusarium* reported here is knowledge gained experimentally by single spore cultures of the variability existing in members of the genus. This knowledge of variability is, of course, only partial, in that one can never hope to learn precisely the exact limits or range of such variability. Therefore, although the foundation for establishing species limits must be gained from a study of variability of the fungi concerned, its application necessarily involves interpretation and judgment on the part of the investigator. For this reason, if for no other, taxonomic systems will never remain fixed. Moreover, as new information is gained in biology, it is bound to influence our classification of biologic units. Indeed, it has now become apparent that the systems of classification available for many fungi have been outmoded as a result of recent research, and that these systems in turn also must be revised, as have been the *Fusaria*, if taxonomy is to serve the worker rather than to obstruct him.

SUMMARY OF SPECIES AND FORMS IN THE GENUS FUSARIUM AND IN THEIR ASCIGEROUS GENERA

Fusarium episphaeria: *Nectria episphaeria*
F. episphaeria f. *coccophila*:

N. episphaeria f. *coccophila*

F. tricinctum

F. tricinctum f. *poae*

F. nivale:

Calonectria nivale

F. nivale f. *graminicola*:

C. nivale f. *graminicola*

F. roseum:

Gibberella roseum

F. roseum f. *cerealis*:

G. roseum f. *cerealis*

F. lateritium:

G. lateritium

F. moniliforme:

G. moniliforme

F. oxysporum

F. oxysporum f. (see 1940 paper)

F. solani:

Hypomyces solani

F. solani f. (see 1941 paper):

H. solani f. (see 1941 paper)

Doubtful Species

F. ciliatum:

Calonectria ciliatum

F. rigidiuscula:

C. rigidiuscula

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIFORNIA

LITERATURE CITED

- BENNETT, F. T. 1933. *Fusarium* species on British cereals: *F. nivale* (Fr.) Ces. Ann. Appl. Biol. 20: 272-290.
- . 1935. *Fusarium* species on British cereals. Ann. Appl. Biol. 22: 479-507.
- BERKELEY, M. J., AND C. E. BROOME. 1859. Notices of British fungi. Ann. Mag. Nat. Hist. 3 ser. 3 p. 376.
- BRICK, C. 1908. Einige Krankheiten und Schädigungen tropischer Kulturpflanzen. Jber. Ver. angew. Bot. 6. 36 p. Berlin.
- COOKE, M. C. 1878. Ravenel's American Fungi. Grevillia 6: 129-146.
- CORDA, A. C. J. 1837. Icones Fungorum hucusque cognitorum. Abbildungen der Pilze und Schwämme. Prag. I. 32 p.
- DESMAZIERES, J. B. H. J. 1848. Seizieme notice sur les Plantes Cryptogames racemement decouvertes en France. Ann. Sci. Nat. Ser. 3 10: 359.
- DICKSON, J. G. 1939. Diseases of cereal and forage crop plants. 259 p. Burgess Publishing Co., Minneapolis, Minn.
- FRIES, E. 1825. Syst. orbis veget. Lundae p. 317.
- GORDON, W. L., AND R. SPRAGUE. 1941. Species of *Fusarium* associated with root rots of the Gramineae in the Northern Great Plains. Plant Disease Reporter 25: 168-180.
- GORDON, W. L. 1944. The occurrence of *Fusarium* species in Canada. I. Canad. Jour. Res., C, 22: 282-286.
- HANSEN, H. N., AND R. E. SMITH. 1932. The mechanism of variation in imperfect fungi: *Botrytis cinerea*. Phytopathology 22: 953-964.
- HANSEN, H. N., AND W. C. SNYDER. 1943. The dual phenomenon and sex in *Hypomyces solani* f. *cucurbitae*. Amer. Jour. Bot. 30: 419-421.
- LINK, H. F. 1809. Observationes in Ordines plantarum naturales. Diss. I. Mag. d. Ges. naturforsch. Freunde. Berlin. 3: 10.
- . 1816. Observationes in Ordines plantarum naturales. Diss. II. Mag. d. Ges. naturf. Freunde. Berlin. 7: 25-45.
- MITTER, J. H. 1928. Studies in the genus *Fusarium*. VII. Saltation in the section *Discolor*. Ann. Bot. 43: 379-409.
- NEES, C. G. 1817. System d. Pilze u. Schwämme. Wurzburg. 38. 329 p.
- OSWALD, J. W. 1942. Taxonomy and pathogenicity of fungi associated with root rot of cereals in California with special reference to the *Fusaria* and their variants. Ph.D. Thesis, University of California, Division of Plant Pathology.
- PECK, C. H. 1903. Report of the State Botanist. 1902. N. Y. State Mus. Bul. 67: 29.
- PETCH, T. 1922. Studies in entomogenous fungi. I. The Nectriae parasitic on scale insects. Brit. Mycol. Soc. Trans. 7: 89-167.
- . 1936. *Gibberella saubinetii*. Ann. Mycol. Berlin 34: 256-260.
- RAILLO, A. I. 1935. Diagnostic estimation of morphological and cultural characters of species in the genus *Fusarium*. (In Russian with English title.) Inst. Zashch. Rast. Trudy Zashch. Rast. ser. 2. Phytopath. 100 p.
- SHELDON, J. L. 1904. A corn mold (*Fusarium moniliforme* n. sp.) Nebraska Agric. Exp. Sta. Ann. Rept. 17: 23-32.
- SNYDER, W. C., AND H. N. HANSEN. 1940. The species concept in *Fusarium*. Amer. Jour. Bot. 27: 64-67.
- , AND ———. 1941. The species concept in *Fusarium* with reference to section *Martiella*. Amer. Jour. Bot. 28: 738-742.
- SPRAGUE, R. 1944. Rootrots of cereals and grasses in North Dakota. N. Dakota Agric. Exp. Sta. Tech. Bul. 332. 35 p.
- TODE. 1791. Fungi Mecklenburg. 2: 21.
- WELLMAN, F. L. 1943. A new species of *Fusarium* causing vascular wilt of tomato. Phytopath. 33: 956-958.
- WIANT, J. S. 1937. Investigations of the market diseases of cantaloups and honey dew and honey ball melons. U. S. D. A. Tech. Bull. 573. 48 p.
- WINELAND, G. O. 1924. An ascigerous stage and synonymy for *Fusarium moniliforme* Jour. Agric. Res. 28: 909-922.
- WOLLENWEBER, H. W., AND C. D. SHERBAKOFF, O. A. REINKING, HELEN JOHANN, AND ALICE BAILEY. 1925. Fundamentals for taxonomic studies of *Fusarium*. Jour. Agric. Res. 30: 833-843.
- , AND O. A. REINKING. 1935. "Die Fusarien." 335 p. Paul Parey, Berlin.

AUXIN AND NITROGEN RELATIONSHIPS IN GREEN PLANTS¹

George S. Avery, Jr., and Louise Pottorf

It was shown in a previous study that auxin is scarcely detectable in the stem tips of nitrogen-starved plants. An increased nitrate supply in the nutrient solution, however, resulted in increased auxin content of the stem-tip tissues (Avery, Burkholder and Creighton, 1937). This earlier work was carried out before auxin extraction techniques had been studied to any extent, and the present report is possible only because of a newly developed method for total extraction of auxin from certain Cruciferae (Avery, Berger and White, 1945). There is a general agreement between the old and the new results,

¹ Received for publication August 25, 1945. Work carried out at Connecticut College.

and the work presented here extends the biochemical side of our knowledge of this problem.

One objective of this study was to determine whether the relative amount of nitrate supplied to growing plants significantly and directly influences the total auxin extractable from its tissues. A second objective was to determine whether any correlation exists between the auxin and nitrogen content of tissues.

MATERIALS AND METHODS.—Kohlrabi seedlings (*Brassica caulorapa* Pasq.) were grown in soil in the greenhouse until they were approximately 15 cm. high. After carefully washing the roots free of soil, they were transplanted to glazed crocks containing pure quartz sand. From this stage until har-

vest, six to nine weeks later, all necessary elements were supplied in known nutrient solutions in which the nitrogen content was varied. The solutions corresponded to 4.0, 1.0, 0.1, 0.01 and 0.00 in the nitrogen series used by Avery, Burkholder and Creighton in a previous study (1937).

During the first five weeks in which the plants were grown in the above solutions, decided differences in size and relative vigor became apparent. Those which were grown in the 4.0 nitrogen solution were not significantly different in size from those grown in 1.0 nitrogen. However, abnormal intumescence-like growths appeared on the older leaves, possibly indicating a toxic condition resulting from an oversupply of nitrogen. In some instances the characteristic kohlrabi stem enlargement commenced prior to harvesting. This enlargement was slightly more pronounced in plants grown in the 4.0 nitrogen solution, as compared to those grown in 1.0 nitrogen. The remaining three groups of plants, supplied with successively less, and no nitrogen, were significantly smaller than those receiving 4.0 and 1.0 nitrogen; the characteristic stem enlargement failed to occur in any of these. All were clearly "starved," but those grown in 0.01 nitrogen, and in no nitrogen, were the smallest and did not differ appreciably in size or appearance from each other. Leaf color varied from medium to bright green in the three groups of plants which received the most nitrogen; those receiving 0.01 nitrogen, or less, were dull yellowish to grayish-green, indicating nitrogen deficiency. Stem color was green in all plants except those grown in 0.01 and no nitrogen; the stems of these were purple. The plants grown in 1.0 nitrogen solution were equivalent in color and growth vigor to those grown in good soil.

It was not possible to secure leaves of equal size from plants grown in the different nutrient solutions because the rate and extent of growth varied with the relative amount of nitrogen supplied. Hence, in selecting leaves from plants grown in the different nutrient cultures, care was taken to choose those which corresponded in age as nearly as possible. Leaves from plants supplied with the highest concentration of nitrogen were dried first, and those supplied successively less nitrogen followed. The growing points from all series were dried at the same time. The actual drying of most samples was accomplished in three to five days, at low temperature and pressure, in an apparatus described by Hays and Koch (1942). Each lot of tissue, when dry, was ground with mortar and pestle and placed in a vacuum desiccator; it was then stored in darkness at room temperature until used.

To extract the auxin, the dried, finely ground tissue was autoclaved in 1 N NaOH for 30 minutes at 120°C., according to the method of Avery, Berger and White (1945). The autoclaved tissue suspension was then centrifuged; the clear supernatant liquid thus obtained was pipetted off, adjusted to approximately pH 6 with hydrochloric acid, and made up in a series of dilutions for auxin assay. Skoog's de-

seeded *Avena* method was used for auxin determinations (cf. Avery, Creighton and Hock, 1939), and all computations of yield were made from *Avena* curvatures in the proportionality range. All results are expressed in "TDC" per gram dry weight of tissue; 100,000 TDC are equivalent to approximately one microgram of 3-indoleacetic acid (Avery, Berger and Shalucha, 1941).

The auxin yields for kohlrabi as reported here are regarded as the total auxin extractable from the tissues. "Total auxin" has been defined as the "free" or water soluble auxin plus the "precursor" (cf. Avery, Berger and Shalucha, 1941). The term "precursor" is used here as in preceding papers to designate a compound which is physiologically inactive in the *Avena* test until converted to auxin by suitable treatment, such as alkaline or acid hydrolysis. Total auxin in green tissues of Cruciferae is generally 20 to 30 per cent free auxin and 70 to 80 per cent precursor (Avery, Berger and Shalucha).

RESULTS.—Check on methods.—In order to obtain maximum yields of total auxin, various means were tested for converting precursor to auxin. First, various lengths of time for autoclaving were tried: Autoclaving for periods of 15 minutes, 30 minutes, one hour, two hours, four hours and over-night (12 to 14 hours) confirmed the observations of Avery, Berger and White (1945), namely, that consistent results in the proportionality range were readily obtainable when the length of heat treatment was 30 minutes.

To determine the effect of strength of alkali on auxin extraction, various lots of tissue were autoclaved in concentrations of NaOH ranging from 0.1 to 1 N. Measurable results in the *Avena* test, i.e., those in the proportionality range, were obtained only when 1 N NaOH was employed. A considerably smaller yield was obtained when 0.1 N NaOH was used, even if the time of autoclaving was extended to one and one-half hours; shorter periods of autoclaving gave very small auxin yields.

To determine the effect of storage on the dried tissue (in a vacuum desiccator in darkness at room temperature), tests were repeated six months after the tissue had been dried. There was no evidence of deterioration in that length of time.

Auxin yield in relation to amount of nitrogen supplied in the nutrient solutions.—Within certain limits of nitrate supply in the nutrient solution, auxin yields from leaves of kohlrabi plants are proportional (table 1). Thus, between 1.0, 0.1 and 0.01, there are ten-fold differences in nitrate concentration in the nutrient solutions, and approximately four-fold differences in auxin content of the leaves of plants grown therein; auxin concentration in leaves, therefore, is clearly related to the nitrogen supply. An oversupply of nitrogen in the nutrient does not further increase the auxin content of the tissues—leaves of 4.0 and 1.0 nitrogen plants, for example, possess about the same amount of extractable auxin.

Stem tips of the 4.0 and 1.0 nitrogen groups con-

TABLE 1. *Auxin yield from leaves and stem tips of kohlrabi, as related to the amount of nitrogen supplied in the nutrient. All auxin yield data are expressed in TDC (total degrees curvature in deseeded Avena test plants); 100,000 TDC equal approximately 1 microgram of indoleacetic acid.*

Relative amounts of nitrogen supplied in nutrient solution to the growing plants	Large (old) leaves			Stem tips		
	Number of tests	Auxin yield in millions of TDC/gm. dry weight (average of all tests)	Control: <i>Avena</i> curvature for indoleacetic acid (10 micrograms per liter)	Number of tests	Auxin yield in millions of TDC/gm. dry weight (average of all tests)	Control: <i>Avena</i> curvature for indoleacetic acid (10 micrograms per liter)
4 N	3	9.7	6.7°	4	51	6.2°
1 N	4	10.0	6.2°	3	55	6.2°
0.1 N	2	2.9	6.5°	5	43 ^a	6.3°
0.01 N	4	0.8	6.5°	3	45	6.5°
0.0 N	4	1.1	6.3°	5	23	5.6°

^a Consistently poor proportionality in five different tests, even though dilutions were clearly in the "proportionality range."

tain about the same amount of auxin; stem tips of plants receiving 0.1 and 0.01 nitrogen, however, are but slightly lower in auxin than those from 1.0 nitrogen plants. Stem tips of plants from the "no" nitrogen group yield about half as much extractable auxin as those from plants receiving ample nitrogen for normal growth.

Thus, the trend is the same in both leaves and stem tips, but it is much less marked in the latter. This is to be expected since the stem tip tends to mobilize all the nitrogen available in the plant, and only in the case of extreme nitrogen deficiency is there a decided drop in auxin production in stem tips. Leaves of plants require ten times as much nitrogen (as stem tips) in the nutrient solution before it is clearly reflected in increased auxin production.

Auxin yield in relation to nitrogen content of the tissue.—Both soluble and total nitrogen determinations were carried out in the Microchemical Laboratory of the California Institute of Technology, through the courtesy of Dr. A. J. Haagen-Smit. There was insufficient tissue of several of the leaf samples, so only stem tips were used for nitrogen determinations. The data are summarized in table 2.

There is no significant difference in the total nitrogen content of the stem tissues of plants grown with "no nitrogen" and "0.01 nitrogen" in the culture nutrient. Both are obviously deficient, yet the small amount of nitrogen present in the 0.01 nutrient is responsible for an increase in soluble nitrogen in the plant tissue. This increase in soluble nitrogen amounts to approximately 50 per cent, but the auxin increase is 100 per cent over "no nitrogen"! With a

further ten-fold increase in nutrient nitrogen there is a very marked increase in both soluble and total nitrogen in the plant tissue, but the corresponding increase in auxin is slight. This means that the critical range of nitrate concentration in the nutrient solution, as far as auxin production by the plant is concerned, is between 0.01 and no nitrogen (tables 1, 2). Thus, the plant produces auxin on much less nitrogen than it takes to affect growth visibly.

Discussion.—The fact that auxin was scarcely detectable in the stem tips of nitrogen-starved *Nicotiana* and *Helianthus* plants (Avery, Burkholder and Creighton, 1937), yet is abundantly available in similarly starved stem tips of kohlrabi in the present study, is undoubtedly attributable to the methods employed. The "diffusion" method of the earlier studies removes only a small amount of the free auxin, whereas the extraction technique removes all the auxin and auxin precursor. Auxin precursor is not auxin, yet in the economy of the plant it constitutes the major share of the total potential auxin. Whether the precursor (defined earlier in this paper) might act as an auxin in plants other than *Avena*, remains to be demonstrated.

It should be pointed out here, as in the earlier study, that auxin concentration varies directly with growth vigor, and that both may be controlled to a greater or less extent by varying the nitrogen supply. There is a low point in nitrogen supply, however, where auxin production occurs, but without an accompanying growth response; moreover, under low nitrogen nutrition (0.01) auxin production oc-

TABLE 2. *Nitrogen content of tissues from stem tips of sand-cultured plants which received widely different amounts of nitrates in the nutrient solutions.*

	Nitrogen series			
	4.0 N	1.0 N	0.01 N	No nitrogen
Auxin content in millions of TDC/gm. dry weight (data from table 1)	51	55	45	23
Soluble nitrogen in per cent, on dry weight basis.....	2.02	1.83	0.74	0.54
Total nitrogen in per cent, on dry weight basis.....	7.53	7.45	2.37	2.81

curs before there is any appreciable accumulation of total nitrogen in the plant.

As regards auxin content of tissue in relation to total nitrogen, it is of interest to recall the work of Riker (1939), who reported that although the auxin content of tomato crown gall tissue was higher than that of normal tomato tissue, there was no significant difference when auxin was expressed in terms of total nitrogen in the two. The same observation holds in a general way in this study, but is of particular interest when concentrations of soluble nitrogen are very low in the tissue. With increase in nitrate in the nutrient solution, the soluble nitrogen is the first to increase in the plant, and accompanying it is a marked increase in auxin.

SUMMARY

Leaves and growing points of kohlrabi plants grown at different levels of nitrogen nutrition were assayed for their total extractable auxin, which is a procedure made possible by a recently developed method for rapid and total extraction from green plant tissue.

The auxin plus auxin precursor in kohlrabi leaves is (within certain limits) dependent upon the amount of nitrate supplied in the nutrient solution. Normal or high nitrate nutrition results in total auxin content of leaves approximately ten times that of nitrogen-starved plants.

As compared with leaves, the stem tips of kohlrabi show but little variation in extractable auxin; normal or high nitrate nutrition results in an auxin content only two times that of nitrogen-starved plants.

Stem tips of plants with adequate nitrogen nutrition contain approximately five times as much auxin as the leaves of such plants.

Soluble and total nitrogen determinations on kohlrabi stem tips show a rough correlation between the concentration of auxin and nitrogen in the tissue, i.e., the results suggest that within certain limits there is no significant difference in auxin yield when expressed in terms of nitrogen content of tissue.

The critical range of nitrate concentration in the nutrient solutions, as far as auxin production by the plant is concerned, is between "0.01" and "no nitrogen." The plant produces auxin on much less nitrogen than it takes to affect growth visibly.

BROOKLYN BOTANIC GARDEN,
BROOKLYN 25, NEW YORK

LITERATURE CITED

- AVERY, G. S., JR., J. BERGER, AND B. SHALUCHA. 1941. The total extraction of free auxin and auxin precursor from plant tissue. *Amer. Jour. Bot.* 28: 596-607.
- , ———, AND R. O. WHITE. 1945. Rapid total extraction of auxin from green plant tissue. *Amer. Jour. Bot.* 32: 188-191.
- , P. R. BURKHOLDER, AND H. B. CREIGHTON. 1937. Nutrient deficiencies and growth hormone concentration in *Helianthus* and *Nicotiana*. *Amer. Jour. Bot.* 24: 553-557.
- , H. B. CREIGHTON, AND C. HOCK. 1939. A low cost chamber for phytohormone tests. *Amer. Jour. Bot.* 26: 360-365.
- HAYS, E. E., AND F. C. KOCH. 1942. An apparatus for vacuum drying in the frozen state. *Science* 95: 633.
- RIKER, A. J. 1939. Growth substances in relation to crown gall. *Rept. Proc. 3rd Intern. Congr. Microbiol.* 1939: 546-548.

POLYPLOIDY, AUXIN AND NITROGEN IN GREEN PLANT TISSUE ¹

George S. Avery, Jr., and Louise Pottorf

THE OBJECT of this study was to determine whether there is a significant difference in the amount of auxin extractable from diploid and tetraploid cabbage, hence, whether auxin content may be related to polyploidy. Earlier, and quite preliminary experiments, employing diploid and tetraploid corn kernels, failed to reveal any evidence of a relationship between polyploidy and auxin content (Avery, Berger and Shalucha, 1942); the present more extensive study of green tissues shows the more vigorous tetraploids to be significantly lower in auxin.

Seeds of diploid and tetraploid cabbage were obtained through the courtesy of Dr. Earl H. Newcomer of the University of North Carolina. The parent seed was *Brassica oleracea* var. *capitata* of the "Hollander" strain, originally obtained from the Ferry Morse Seed Company. The tetraploid line was produced by colchicine treatment as described by Newcomer (1943).

The seeds were planted in soil, where the young

seedlings were allowed to remain until they were approximately 12 to 15 centimeters high. At this stage they were transplanted to glazed crocks of pure quartz sand and watered with a full nutrient solution (Avery, Burkholder and Creighton, 1937) until time of harvest. The tetraploid cabbage was more vigorous from the start of germination. It developed more rapidly than the diploid when both were grown under the above conditions, and it had both more and larger leaves at the time the experiment was terminated.

The growth period was brought to a close when the plants were beginning to form heads, approximately two months after the seeds were sown. Leaves of medium size were removed from each plant and dried at low temperature and pressure in an apparatus described by Hays and Koch (1942); stem growing points with a few young leaves were then excised and dried. The actual drying time for each lot of tissue was from three to five days. Diploid leaves were dried before tetraploid; the stem tips of both

¹ Received for publication August 25, 1945.

TABLE 1. *Auxin and nitrogen^a determinations on leaves and stem tips of diploid and tetraploid cabbage.*

Plant material assayed	Number of tests	Auxin yield in millions of TDC/gm. dry weight	Control: <i>Avena curvatura</i> for indoleacetic acid (10 micrograms/liter)	Total nitrogen in per cent (dry weight basis)	Soluble nitrogen in per cent (dry weight basis)
Diploid leaves	5	18.5	10.9°	3.35	0.25
Tetraploid leaves	6	6.8	8.9°	3.77	0.74
Diploid stem tips	4	240	9.6°	5.01	... ^b
Tetraploid stem tips	5	143	9.5°	5.56	1.07

^a Nitrogen determinations carried out in the Microchemical Laboratory of the California Institute of Technology.

^b Insufficient tissue for soluble nitrogen determination.

were dried simultaneously. In each case the dry weight was approximately ten per cent of the fresh weight of the tissue. Each lot of tissue, when dry, was ground with mortar and pestle and stored at room temperature in darkness in a vacuum desiccator. The tissue samples were obtained from 16 diploid and 36 tetraploid plants.

The method of extracting auxin and auxin precursor from the dried finely ground tissue of cabbage was identical with that described in the immediately preceding paper in this issue of the American Journal of Botany. The assay methods, expression of yields, etc., are likewise the same as in the preceding paper. The auxin yields as reported here are regarded as the total auxin and auxin precursor extractable.

RESULTS.—Auxin yield from diploid and tetraploid cabbage.—In preparation for extraction, all samples of tissue in alkali were made up for heat treatment by suspending 40 mg. of dried tissue in 1 gm. of 1 N sodium hydroxide; thus, TDC are based on 1:25 dilution. In all instances the diploid cabbage gave higher auxin yields than the tetraploid (table 1).

It may be seen that leaves of the diploid cabbage give an auxin yield more than twice that of the tetraploid leaves, per unit dry weight; the auxin yield from stem tips of diploid plants is almost twice that of the tetraploids.

Auxin yield in relation to nitrogen content of tissue.—Total nitrogen is somewhat lower in diploid than in tetraploid leaves, yet auxin content is more than double that of the tetraploid. Soluble nitrogen also bears an inverse relationship to auxin content. Furthermore, total nitrogen is lower in diploid than in tetraploid stem tips, and here again, lower nitrogen is accompanied by distinctly higher auxin content. These results are the converse of those reported in the preceding paper—where one genetic line of kohlrabi was grown under varying conditions of nitrogen nutrition.

DISCUSSION.—In the preceding study there was evidence to indicate a direct relationship between the auxin and nitrogen content of tissues (Avery and Pottorf, 1945). That study was made on a single variety of kohlrabi, and the only factor varied in

the experiment was the nitrate in the nutrient solution in which the plants were grown.

In the present study, there was genetic variation in the experimental material (diploid vs. tetraploid plants of cabbage), but the nutrient solution was the same throughout. Thus, the fact that auxin content of the diploid tissue is considerably higher than that of the tetraploid, while nitrogen content is about the same for both, indicates a definite relationship between polyploidy and auxin content of cabbage—which is not associated with nitrogen.

Gustafson (1944) has reported that tetraploid marigolds are lower in auxin content than the diploids, though his data leave much to be desired.

Barr and Newcomer (1943) found tetraploid cabbage lower in soluble nitrogen than the diploid, the opposite of the findings in this study.

SUMMARY

Leaves and stem tips (with very young leaves) of diploid and tetraploid green cabbage were assayed for their total extractable auxin and auxin precursor, a procedure made possible by a recently developed method for rapid and total extraction from green plant tissue. Stem tips contain 14 to 20 times as much auxin as the leaves, per gram dry weight of tissue, and diploids yield two or three times as much auxin as the tetraploids.

Nitrogen determinations on leaves and stem tips show little difference between diploids and tetraploids, thus the wide differences in auxin content of diploid and tetraploid cabbage are not related to nitrogen in the tissue.

BROOKLYN BOTANIC GARDEN,
BROOKLYN, NEW YORK

LITERATURE CITED

- AVERY, G. S., JR., J. BERGER, AND B. SHALUCHA. 1942. Auxin content of maize kernels during ontogeny, from plants of varying heterotic vigor. *Amer. Jour. Bot.* 29: 765-772.
- , P. R. BURKHOLDER, AND H. B. CREIGHTON. 1937. Nutrient deficiencies and growth hormone concentration in *Helianthus* and *Nicotiana*. *Amer. Jour. Bot.* 24: 553-557.
- , AND L. POTTORF. 1945. Auxin and nitrogen relationships in green plants. *Amer. Jour. Bot.* 32: 666-669.

- BARR, C. G., AND E. H. NEWCOMER. 1943. Physiological aspects of tetraploidy in cabbage. *Jour. Agric. Res.* 67: 329-336.
- GUSTAFSON, F. G. 1944. Growth hormone studies of some diploid and autotetraploid plants. *Jour. Hered.* 35: 269-272.
- HAYS, E. E., AND F. C. KOCH. 1942. An apparatus for vacuum drying in the frozen state. *Science* 95: 633.
- NEWCOMER, E. H. 1943. An F_2 colchicine-induced tetraploid cabbage and some comparisons with its diploid progenitor. *Jour. Elisha Mitchell Sci. Soc.* 59: 69-72.

NEUROSPORA. I. PRELIMINARY OBSERVATIONS OF THE CHROMOSOMES OF *NEUROSPORA CRASSA*¹

Barbara McClintock

THE PRESENT report on the chromosomes of *Neurospora crassa* represents the results of observations which were confined to a period of ten weeks in the biological laboratories of Stanford University. The purpose of this study was to obtain some knowledge of nuclear and chromosome behavior in normal and mutant strains. The author realizes that no single phase of these investigations could be adequately studied in so short a period of time. Because of the interest in *Neurospora* as genetic material, a summary of some of these observations will be given at this time.

The observations were confined to the nuclei and chromosomes in the ascus, from fertilization to spore formation. Union of two haploid nuclei occurs in the young ascus. This is followed by a simultaneous enlargement of the ascus and fusion nucleus. During this growth period, the chromosomes in the fusion nucleus enter into meiotic prophase activities, including homologous association of chromosomes, elongation of chromosomes, chiasmata formation and contraction until typical metaphase I bivalents are produced. Although the consequences of this meiotic prophase activity are essentially similar to those observed in many other organisms, the timing of chromosome synapsis and elongation is dissimilar and is of some theoretical interest. The two meiotic mitoses follow in rapid succession leading to the formation of four haploid nuclei. In essential details and accomplishments, the chromosome and nuclear behavior in these two divisions is typical of meiosis in general. Particular details, however, are of interest to the cytologist. Each of the four haploid nuclei in the now greatly enlarged ascus undergoes a typical equational mitosis resulting in a row of eight haploid nuclei. Associated with each nucleus is a centriole which has become greatly enlarged during the meiotic and first post-meiotic mitoses. Fibers emerging from each centriole extend and encircle the cytoplasm surrounding each nucleus. This process initiates wall formation and the cutting out of

eight independent ascospores. Shortly after the spore walls are differentiated, the nucleus in each spore undergoes an equational mitosis. The ascospore continues to maturity with the two resulting nuclei.

METHODS.—Approximately seven days (at 25°C.) after inoculation of an agar slant with the two sex strains, *A* and *a*, perithecia were present containing numerous asci in various stages of pre-fertilization, fertilization, meiosis and spore formation and development. These perithecia were removed from the slant and placed in a drop of staining solution. With the bent end of a needle, pressure was applied to the perithecial wall. When this pressure was properly exerted, the asci within the perithecium were forced out through the ostiole. They usually emerged as a single mass. The perithecial wall was removed and a cover slip placed over the drop. The slide was then gently heated. Several methods of staining were attempted such as aceto-orcein, aceto-carmin, propionic-orcein, lacto-orcein and acetic-lactic-orcein combinations. After many trials, it was realized that the genetic strain being utilized had much to do with the success of the staining procedure. A cross of two particular wild-type strains always gave excellent results, whereas other strains gave moderate or consistently poor results. In general, aceto-orcein was the most reliable chromosome stain but the nucleoli were not differentiated. When, in any particular aceto-orcein preparation, it was necessary to observe the nucleoli, aceto-carmin was subsequently run under the cover slip. The nucleoli, taking up the carmin stain, were then clearly visible.

CHROMOSOME NUMBER.—The haploid chromosome number in all the examined strains of *N. crassa* was seven. This number does not agree with that given by Lindegren and Rumann (1938) for *N. crassa* (six to nine chromosomes) nor that given by Colson (1934) for *N. tetrasperma* (six chromosomes). Seven haploid chromosomes had previously been observed (Dr. E. A. Weaver and author, unpublished) in a strain of *N. tetrasperma* supplied by Dr. B. O. Dodge. The author is indebted to Dr. G. W. Bohn, a former graduate student of the University of Missouri, for calling her attention to the *Neurospora* chromosomes. He observed seven haploid chromosomes in his excellent aceto-carmin preparations of *Neurospora* sp.

CHROMOSOME SIZE.—The lengths of the chromo-

¹ Received for publication August 28, 1945.

The author wishes to express appreciation to Dr. G. W. Beadle for presenting the opportunity for these studies and for the many kindnesses he has shown. Much credit should be given to Mrs. Mary B. Houlahan, Dr. Herschel K. Mitchell and Dr. Lotti Steinitz for their interest and collaboration in the chromosome studies, in selecting and supplying the mutant and wild-type strains and for various helpful suggestions regarding techniques.

somes were measured at various stages from pre-synapsis in the zygote nucleus to the metaphase of the division in the ascospore. The longest chromosome is approximately 2.7 times the length of the

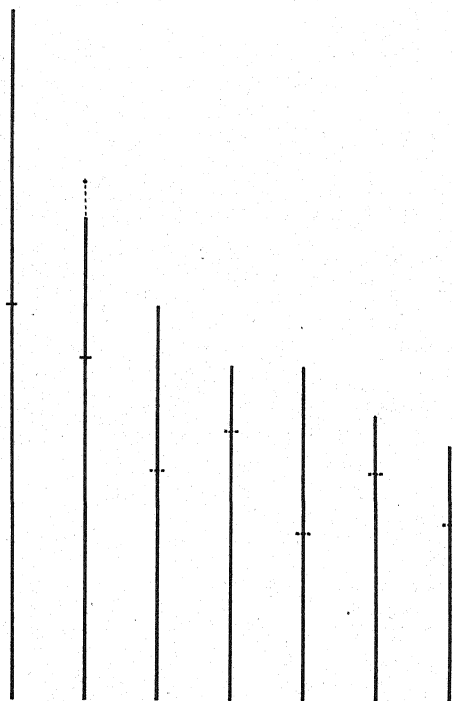


Fig. 1. Diagram illustrating the relative lengths of the seven chromosomes of *Neurospora crassa*. The cross lines indicate the positions of the centromeres; these are reasonably correct for the two longest chromosomes. The determination of the positions of the centromeres in the other chromosomes needs further confirmation; the assigned positions (broken cross-lines) should be considered only as tentative. The separation of the minute satellite from the main segment of the short arm of chromosome 2 is indicated by the dashed line.

shortest chromosome. Since the relative lengths of all chromosomes are maintained throughout the nuclear cycles, measurements will be mentioned only for this longest chromosome. At the end of the pachytene, this chromosome may attain a length of approximately 15 microns. At metaphase of the third division in the ascus, it may be approximately 2.5 microns long. At the metaphase of the division in the ascospore, it may be only 1.5 microns long. The chromosomes of the hyphal nuclei were not examined. In contrast to the relatively large size of the nuclei in the ascus and the ascospores, the hyphal nuclei are very minute. It is probable that the metaphase chromosomes they form are likewise very minute.

RELATIVE LENGTHS OF THE CHROMOSOMES.—Measurements of the relative lengths of the chromosomes were most satisfactorily obtained from nuclei in late pachytene. The chromosomes are then at their maximum extension (see below). Although all seven chromosomes were drawn and measured in only a few

meiotic prophase nuclei, the relative lengths of the chromosomes were consistent within each nucleus. Figure 1 illustrates the relative lengths of the seven chromosomes as computed from these measurements.

MORPHOLOGY OF THE CHROMOSOMES.—*Centromere positions.*—The centromere position was adequately determined only for the two longest chromosomes. The analysis of centromere positions was suspended temporarily because it was thought that one of the smaller pairs of chromosomes might be heteromorphic. If this were true, two sets of chromosome morphologies with respect to centromere positions, would have to be considered. The presence of a heteromorphic pair was not confirmed in subsequent examinations which were confined mainly to a cross between two particular wild-type strains. Whether a heteromorphic pair is present or could be identified in crosses of other wild-type strains remains to be determined. Due to the pressure for other determinations, no time was taken to renew the studies of centromere positions. In order to convey some idea of centromere positions in the complement as a whole, the tentative positions that had been assigned to chromosomes 3 to 7 before this analysis was suspended, are included in figure 1.

The nucleolus chromosome.—The second longest chromosome (chromosome 2) possesses a nucleolus organizer close to the end of its short arm. Consequently, there is a very minute satellite. The nucleolus organizer functions in the usual manner and develops a nucleolus in each telophase nucleus.

Chromomere patterns.—At late pachytene, each chromosome shows a distinct chromomere pattern. The pattern for any one chromosome is constant. The chromomeres have various sizes and shapes. They are separated by thinner strands of chromatin but are not spaced equally along the chromosome. The smaller chromosomes have only a few distinct chromomeres (five to six or seven), whereas the longer chromosomes have correspondingly more. No attempt was made in this preliminary study to map the chromomeres of each chromosome. However, these distinctive chromomere patterns could be useful in identifying individual chromosomes at pachytene. No knobs were recognized in these chromosomes. Centromeres could not be identified with certainty in the orcein stained preparations of pachytene.

Heterochromatin.—Heterochromatic segments of chromosomes were not recognized as such in the pachytene chromosomes. However, the presence of heterochromatin was detected in the telophase nuclei following the second meiotic mitosis and in the resting nuclei of the one- and two-nucleated ascospores. It could also be observed in the hyphal nuclei. There are two main segments of heterochromatin. They are located adjacent to a centromere. It has not been determined whether these two recognized segments lie adjacent to the centromere on opposite arms of one chromosome or whether they are parts of two separate chromosomes. Congression of the centromeres in late anaphase of division III,

and in the spore division, results in the formation of a somewhat pear-shaped resting nucleus. The centromeres of all seven chromosomes lie in the apex of this pear-shaped nucleus. Here, also, are found the two heterochromatic bodies lying so close together that they suggest a single dumb-bell shaped structure. It is believed, however, that they have not fused to form a single chromocenter but are forced close to one another by the intimate spacial association of all seven centromeres. Extensive observations have not been made of these two heterochromatic bodies nor has an attempt been made to identify the chromosome or chromosomes involved.

NUCLEAR FUSION, CHROMOSOME SYNAPSIS AND THE SUBSEQUENT ELONGATION OF THE SYNAPSED CHROMOSOMES.—Fusion of two haploid nuclei to form the zygote occurs in the very young ascus. Illustrations of the appearance of the ascus at this stage are given by Colson (1934). At the time of fusion, the chromosomes of each nucleus appear to be in a resting stage and a nucleolus is present in each. Following nuclear fusion, the chromosomes contributed by each nucleus undergo what appears to be a typical prophase contraction until, in some strains, the chromosomes may be almost as short as those of the metaphase of the third division in the ascus. No obvious doubleness of the chromosomes was observed, however. During this period, fusion usually occurs between the nucleoli contributed by each nucleus. At the end of the contraction period, the two haploid sets of chromosomes lie, roughly, at opposite sides of the zygote nucleus. In this highly contracted state, the homologous chromosomes enter into the synaptic phase of the meiotic cycle. In the early synaptic phase, many nuclei were observed with some homologous chromosomes lying adjacent to one another but not in actual physical contact. It is not clear whether this early stage in the association process is the consequence of a directed migration of homologues toward one another or whether this stage is reached following random movements of the chromosomes within the nucleus. Possibly the movements of the chromosomes could be followed in tissue cultures of the living asci. It is of considerable theoretical interest to determine the range of the force of synaptic attraction. The actual physical association of the chromosomes usually begins at one or both ends and continues along them. In many zygote nuclei, synapsis is completed for some pairs of chromosomes before the members of other pairs have come in contact. Soon, many nuclei show seven short, but completely synapsed, bivalent chromosomes. (Most of the detailed observations of the synaptic phase were confined to asci resulting from the cross of two wild-type strains (Emerson 5256A \times Chilton-a). In crosses of some other strains, synapsis appears to occur when the chromosomes are less contracted.) Following completion of synapsis and possibly during this period, the chromosomes commence their elongation. This is possibly an uncoiling process for in some early post-synaptic nuclei, the elongating chromosomes ap-

peared to possess compressed gyres. This elongation process continues until the chromosomes have reached their full extension. At this stage, the chromosomes are essentially similar in appearance to the pachytene chromosomes of many other organisms. The term "pachytene" has therefore been used. Although homologous chromosomes lie side by side at late pachytene, they are often not closely appressed. Often, there is little or no relational coiling of the two homologues around one another. During the period from zygote formation to late pachytene, the volume of the nucleus and nucleolus increases steadily. In all post-synaptic stages, the volume of the nucleus is very much greater than that of the chromosomes. Consequently, the chromosomes are widely spaced within the nucleus. During all these stages, the chromosome 2 bivalent remains attached to the nucleolus by the organizer regions. At pachytene, the organizer regions of the two homologues may diverge slightly from one another; the satellites may be some distance removed from them.

CHROMOSOME BEHAVIOR FROM DIPLOTENE TO THE THIRD DIVISION IN THE ASCUS.—At diplotene, a wide separation occurs between parts of a bivalent chromosome but the individual chromatids were difficult to follow. Coiling commences at diplotene and the contraction of the chromosomes is very rapid. At diakinesis, typical chiasmata may be seen. No attempt was made to count chiasmata but it is possible to do so at this stage. The chromosomes continue contraction to form typical metaphase I bivalents with terminal and interstitial chiasmata. Although the nucleolus becomes smaller during the pre-metaphase stage, it does not disappear. Chromosome 2 remains attached to the nucleolus by its organizer region. Anaphase I separation of the chromosomes appears to be essentially typical except for the nucleolus. This may be dragged toward one pole or stretched between the poles because the nucleolus organizers of one or both of the dyad chromatids of chromosome 2 have not been released from their attachment to the nucleolus. The nucleolus becomes detached before telophase sets in and may subsequently be seen in the cytoplasm of the ascus. At telophase I (and likewise telophase II and III) the centromere regions of all the chromosomes form an aggregate that lies at the apex of a distinct protrusion of the nucleus (the beak: Dodge, 1927). No true resting nucleus is formed. Instead, the chromosomes uncoil and the individual arms of each chromosome extend into an elongated nucleus. A new nucleolus is produced by and remains attached to the nucleolus organizers of chromosome 2. Prophase II proceeds by contraction of these elongated chromosomes until the two dyads of each chromosome form very short, parallel rods, each showing a conspicuous centromere region. Metaphase and anaphase II proceed normally. At telophase II, the chromosomes, whose centromere regions are again aggregated at the apex of the beaked nucleus, uncoil and the two arms of each chromosome extend into the nucleus as individual strands and remain in this con-

dition until the following prophase. The extent of elongation of the chromosomes appears to be similar to that of late pachytene. In each nucleus, a new nucleolus is formed at the position of the nucleolus organizer of chromosome 2. Prophase III proceeds by contraction of the arms of the chromosomes. Because the chromosomes maintain their previous telophase orientation (Js and Vs) during this contraction, the prophase of division III is a satisfactory stage for observing the relative lengths of the arms of a chromosome. Metaphase and anaphase of the

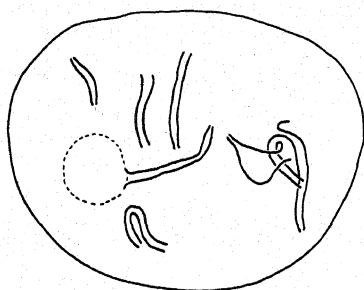


Fig. 2. Outline drawing of the synapsed chromosomes in an ascus heterozygous for T 4637. There are five bivalents and a synaptic configuration of four chromosomes. The nucleolus is outlined by a dashed line. The minute satellites of the pair of nucleolus chromosomes were not detected in this figure.

third division proceed as a typical equational mitosis. The telophase of this division is followed by a condition of the nucleus resembling a resting stage. Shortly after spore delimitation, a mitosis occurs in each ascospore. This is likewise a typical equational mitosis. In essential details, divisions I and II are typically meiotic. Division III is essentially a somatic mitosis except that the chromosomes retain their identity from the telophase of division II to the prophase of division III. It would be of interest to determine the time of effective splitting of the chromosomes for this division.

RECIPROCAL TRANSLOCATIONS.—In the Stanford laboratory, many mutants have been obtained following x-ray and ultra-violet irradiations. Chromosomal abnormalities could likewise be expected to occur from such treatments. Three irradiation-induced mutants (4637, 44105 and 45502) whose genetic behavior suggested the presence of some chromosomal abnormality, were selected and crossed to normal wild-type strains. The chromosomes were examined in the asci developing from these crosses. In all three cases, the ascus nuclei were heterozygous for a translocation between two non-homologous chromosomes. In the limited time available, it was not possible to make an intensive study of each translocation. Nevertheless, some observations and interpretations based on these studies will be mentioned.

Translocation 4637.—Figure 2 represents an outline drawing of late pachytene chromosomes in an ascus nucleus developing from the cross of the albino mutant strain 4637 by a wild-type strain. There are five normal bivalents and a synaptic configuration

of four chromosomes (right). In these nuclei, homologous associations of all parts of the four chromosomes were not always accomplished. Unsynapsed segments, as illustrated in figure 2, were frequently observed. Sometimes, at pachytene, the four chromosomes were present as two "bivalents" with synaptic associations only between their respective homologous parts. At diakinesis and metaphase I, either a ring of four chromosomes, a chain of four chromosomes or two "bivalent" chromosomes were observed.

Translocation 44105.—Relatively few observations were made of the translocation introduced by mutant strain 44105. These were limited to a few figures of diakinesis and metaphase I. A ring of four chromosomes was observed in one metaphase I figure. In several others, one or more of the chromosomes were present as univalents. In two figures, all four chromosomes were present as univalents. No pachytene configurations were observed.

Translocation 45502.—The reciprocal translocation introduced by mutant strain 45502 involved a very unequal exchange of segments of two non-homologous chromosomes. The breaks appear to have occurred close to the end of the long arm of chromosome 1 and close to the centromere in the long arm of one of the chromosomes with a sub-terminal centromere. This translocation could serve several purposes which will be outlined below.

Estimates of the types of disjunction of chromosomes in asci heterozygous for T 45502.—Because of the small size of the metaphase and anaphase I chromosomes in *Neurospora*, it would be very laborious to determine by direct observations the modes of disjunction of the four chromosomes involved in translocation configurations. An examination of the eight-spored asci developing from asci whose fusion nuclei were heterozygous for translocation 45502 has suggested a possible method of estimating these disjunctions. In most organisms, a two-by-two disjunction of the four chromosomes of an interchange complex usually occurs at anaphase I. In organisms having the *Oenothera* type of disjunction, alternate chromosomes in a ring or chain of four or more chromosomes go to the same pole at anaphase I. In maize, *Pisum*, etc., the four chromosomes of a ring usually disjoin so that two members go to one pole and two to the opposite pole. In these forms, alternate disjunctions occur in some cells. In other cells, however, two adjacent members of the ring or chain of four chromosomes may go to the same pole. When a heterozygous translocation is present in *Neurospora*, do the chromosomes disjoin according to the *Oenothera* pattern or do disjunctions follow the maize and *Pisum* pattern? The analysis given below suggests that the disjunctions in *Neurospora* are similar to those observed in maize and *Pisum*.

Although the exact position of breakage in the two chromosomes has not been determined, a diagram illustrating the type of synaptic configuration to be expected in asci heterozygous for T 45502 is given in figure 3. If no crossing over occurs in either re-

gion *a* or *b*, figure 3, alternate disjunctions ($1+4 : 2+3$, fig. 3) of the four chromosomes at anaphase I when a ring or a chain is present, or the counterpart type of disjunction when two "bivalents" are present, should produce an ascus with eight normal spores (Type I ascus, fig. 3). In this case every spore would receive a full genomic complement, four with the normal chromosomes ($1+4$, fig. 3), and four with the translocation chromosomes ($2+3$, fig. 3). When two adjacent chromosomes of this complex pass to the same pole at anaphase I, all eight of the resulting spores in an ascus would be deficient for some part of the genomic complement. There are two possible types of adjacent disjunctions, those which result from disjunctions of homologous centromeres ($1+2 : 3+4$) and those which result from non-disjunction of homologous centromeres ($1+3 : 2+4$). The former will be called adjacent I disjunction, the latter, adjacent II disjunction. Following adjacent I disjunctions, four of the spores (with $1+2$) would be deficient for

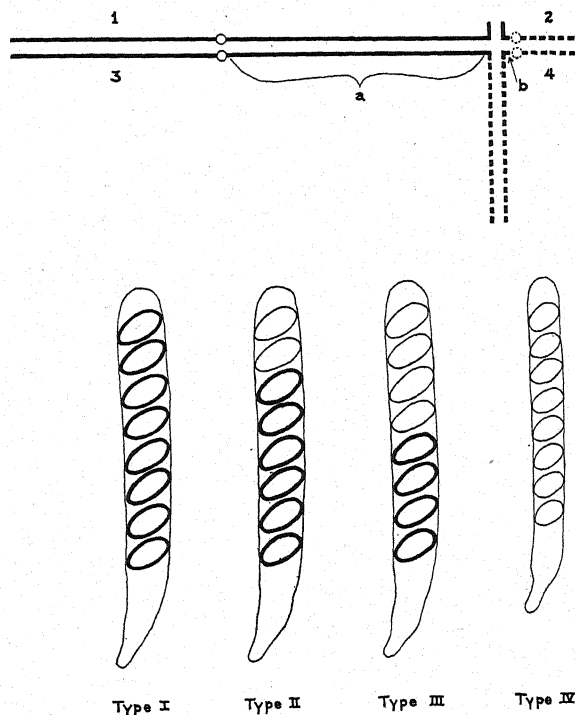


Fig. 3. *Upper*. Diagram illustrating the complete synaptonemal association of two normal and two translocated chromosomes in an ascus heterozygous for a very unequal reciprocal translocation. Chromosomes numbered 1 and 4 represent the normal chromosomes; chromosomes numbered 2 and 3 represent the translocation chromosomes. The centromere in each chromosome is represented by a circle. *Lower*. Diagrammatic representation of the types of eight-spored asci resulting from several types of disjunctions of the four members of the synaptic complex (see text for explanations). The heavily outlined spores are normal in appearance; the lightly outlined spores are visibly defective in appearance. (In the observed material, the type IV ascus is considerably more defective than the diagram suggests.)

nearly all of the long arm of one chromosome. In contrast, the four spores with $3+4$ would be deficient only for a small segment of the genomic complement. Comparable studies in maize have shown that spores with deficiencies of large segments of the genomic complement are defective in appearance, whereas spores with small deficiencies may be normal in appearance, especially in the early developmental stages. If the response in *Neurospora* is similar, it could be expected that the spores with $1+2$ would be defective in appearance, whereas those with $3+4$ may be normal in appearance, especially in the young eight-spored asci. If this occurs, adjacent I disjunctions would give rise to asci with four adjacent defective spores and four adjacent spores which appear to be normal (Type III ascus, fig. 3). If adjacent II disjunctions occurred, all eight spores would be deficient for relatively large segments of the genomic complement. All eight spores could be expected to show visible evidence of the deficiencies (Type IV ascus, fig. 3).

On the supposition that the spores with $1+2$ are defective in appearance and those with $3+4$ are normal in appearance, a fourth type of eight-spored ascus could be anticipated. This would be formed whenever a crossover had occurred between the centromere and the position of break (regions *a* or *b*, fig. 3). In this *Neurospora* translocation, such a crossover is probably confined almost entirely to the long segment of region *a*. Few crossovers would be expected to occur in the very short *b* segment. Studies of the disjunction of the four chromosomes involved in a heterozygous translocation in maize have revealed that whenever a crossover has taken place between the centromere and the position of breakage, homologous centromeres will pass to opposite poles at anaphase I (McClintock, unpublished). If this crossover-disjunction relationship likewise applies to *Neurospora*, the resulting eight-spored asci should possess four spores with normal genomic complements (two with $1+4$ and two with $2+3$), two normal appearing spores with the short deficiency ($3+4$) and two adjacent defective spores with the longer deficiency ($1+2$) (Type II ascus, fig. 3).

As table I indicates, four main types of asci corresponding to types I to IV, figure 3, were observed. The eight-spored asci were all relatively young, as the counts were made from slides prepared for chromosome studies. In each count, the relative frequencies of the types of asci are similar. Observations of the spore appearances in mature asci were made by Mrs. Mary B. Houlihan. She found that the asci with two very defective spores had, in addition, two immature appearing spores plus four normal appearing spores. These should be type II asci; the spores with the short deficiency, ($3+4$), not distinguishable in the young stage from spores having a normal genomic complement, are now detectable because of their slower rate of maturity.

It should be stated that in ascus type II, the two adjacent defective spores occupied any one of the four possible positions in the ascus, with approxi-

TABLE 1. Frequencies of asci with normal and defective spores in six preparations. The zygote nuclei were heterozygous for a translocation associated with mutant strain 45502.^a

Type I ascus	Type II ascus 2 defective sister spores and 6 normal spores	Type III ascus 4 adjacent defective spores and 4 normal spores	Type IV ascus
All 8 spores normal			All 8 spores defective
20	50	28	17
24	41	34	8
37	85	32	18
37	58	30	17
54	97	50	16
25	50	24	17
Totals			
197	381	198	93 ^b

^a Record was made of 17 asci with normal and defective spore orientations other than types II and III of this table. See text for description.

^b In making these slides for chromosome studies, many of the asci of types I to III were broken and their spores scattered. Only non-broken asci were scored. Type IV asci were not so readily broken. Thus, the figure for type IV probably is relatively too high.

mately equal frequencies. This is to be expected if the orientation of the chromosomes at metaphase I and II is at random with respect to the long axis of the ascus. Likewise, in ascus type III, the four adjacent defective spores occupied positions either at the base or the tip of the ascus.

On the basis of the explanation of the types of eight-spored asci given above, the following conclusions may be drawn: (1) When no crossing over occurs between the centromere and the point of interchange, alternate and adjacent I disjunctions will occur equally frequently (types I and III, table 1). (2) Adjacent II disjunctions are relatively infrequent (type IV, table 1; see accompanying footnote). (3) A crossover occurs in the longer chromosome between the centromere and the position of breakage in approximately half of the ascus nuclei (type II, table 1). It is fully realized that these studies are only preliminary and require further investigation. Nevertheless, the author wishes to emphasize the possible usefulness of this type of analysis as a complement to the cytological observations.

A POSSIBLE METHOD FOR DETERMINING THE FREQUENCY OF TRANSPOSITION OF SPORES.—In many genetic analyses, the order of the spores in an ascus is of prime importance. The eight spores in an ascus are linearly arranged and are assumed to reflect the orientation of the nuclei and spindles in the three preceding divisions in the ascus. Following division I, the two resulting nuclei are some distance apart in the ascus cytoplasm. The spindles they form are parallel to the long axis of the ascus. Thus, following the second division, four nuclei are present, the upper two derived from one nucleus, the lower two derived from the second nucleus. Maintaining their respec-

tive positions in the ascus cytoplasm, each nucleus again divides and a row of eight free nuclei are formed. It is not until then that walls appear cutting out the eight spores. If no disturbances have occurred in the arrangement of the nuclei and spindles during the free-nucleated stage, the position of each spore reflects its origin with respect to the three preceding divisions. Lack of wall formation following divisions I and II in the ascus is a distinct disadvantage. Irregularities in spindle orientation or transposition of the usual order of two or more of the free nuclei will lead to linear arrangements of spores which do not reflect their origin in the previous spindles. Irregularities of this sort are known to occur and it is important for some investigations to determine their frequencies.

The reciprocal translocation in mutant strain 45502 or a chromosomal abnormality giving similar types of recognizable defective spores, might be useful for estimating the frequency of occurrence of aberrant alignments of some of the spores in an ascus. In addition to the ascus types recorded in table 1, there were 17 asci with normal and defective spore orientation other than types II and III. If, after the second meiotic mitosis following an adjacent I disjunction described above, the two inner nuclei (with 1+2 and 3+4, respectively) exchanged positions, the spore alignment would not be type III. Instead, two adjacent normal appearing spores (with 3+4) would be inserted between the two sets of sister defective spores (with 1+2). Seven of the 17 aberrant asci were of this type. If, following division III in an ascus destined to be of type II, two non-sister nuclei exchanged positions, a spore alignment other than type II could appear. This would occur if one of these nuclei possessed the long deficiency (1+2) which gives rise to the defective appearing spores. In these asci, the two defective appearing spores would now be separated by a normal appearing spore. Five such asci were observed among the 17 aberrant asci mentioned in the footnote to table 1. These observations are not considered adequate for estimating the frequency of nuclear displacements. More study needs to be given to the aberrant asci to determine whether displacement of spores may occur after spore delimitation through rough handling, or whether additional disturbances, such as aberrant chromosomal behavior, are contributing factors. Because of the significance of aberrant alignment of spores in genetic investigations, it was considered worth while to mention a possible rapid method of estimating their frequencies.

CONCLUSIONS.—The usefulness of fungi as genetic material has been well demonstrated in recent years. To interpret properly the results of many genetic investigations, it is either advantageous or necessary to know the accompanying chromosomal conditions. On the basis of this brief study of *Neurospora* chromosomes, the author anticipates that some fungi may prove to be adequate and in some respects superior cytogenetic material. A review of the literature sug-

gests that some forms may be distinctly superior to *Neurospora* for studies of chromosome behavior, particularly of those stages from fertilization to the first meiotic metaphase. Forms with two haploid chromosomes, one of which is associated with the nucleolus, might prove to be very satisfactory in following the stages and motions of the chromosomes during synapsis, in studying the consequences of various chromosomal rearrangements and for other studies involving the meiotic prophase periods. In ascomycetes, the ease of isolation of the asci, the abundance of asci and the relation of size to stage in meiosis should recommend this material for tissue cultures when it is desired to observe the chromosomes during the meiotic stages in living nuclei.

The haploid chromosomal complement of *Neurospora crassa* is similar in its organization to that observed in many organisms. Each of the seven chromosomes may be identified not only by its relative length, the position of its centromere, but also by the constancy of its internal organization as exhibited by chromomere patterns in the meiotic prophase. One chromosome of the haploid complement possesses a nucleolus organizer which functions just as it does in other organisms. Because of the location of the nucleolus organizer near the end of one arm of this chromosome, there is a minute satellite. Even the coiling and uncoiling processes leading to contraction and expansion of the chromonema appear to be similar to that observed in many other organisms. No distinctively unique features of chromosomal organization were recognized. The presence of translocations between non-homologous chromosomes following irradiation treatment and the behavior of these translocated chromosomes in the meiotic stages of heterozygous asci likewise are indicative of the orthodox organization of the *Neurospora* nuclei and chromosomes.

It has been observed that the behavior of the chromosomes in the first two mitoses in the ascus results in the formation of four haploid nuclei whose chromosomes have been subjected to the processes common to meiosis in general: synapsis of homologous chromosomes, chiasma formation, and typical anaphase I and II disjunctions and segregations of chromatids. The synaptic period, however, is distinctly atypical. In many organisms, synapsis is initiated in the meiotic prophase when the chromosomes are much extended. In the *Neurospora* strains most extensively studied, this period occurs when the chromosomes are contracted, short rods simulating late prophase chromosomes. Elongation of the chromosomes to their maximum meiotic prophase extension takes place after the chromosomes have become homologously associated throughout their lengths. If the chromonema within each chromosome at the time of synaptic attraction and association is tightly coiled, the homologous associations along the chromosomes cannot be equally intimate. Other cases of synaptic attraction of condensed chromosomes have been described but *Neurospora* offers rather unique opportunities for studying this process.

The centriole has not been considered in previous sections of this report, but it deserves a brief mention because of its steady enlargement during the interphase stages of the divisions in the ascus, its relation to the centromeres during this enlargement, as well as its previously known function in initiating spore wall formation (Harper, 1905; Dodge, 1927; Wilcox, 1928). As mentioned previously, the interkinetic nuclei following divisions I, II and III are somewhat pear-shaped because of a decided protrusion or "beak." The centromeres of all chromosomes form a compact aggregate at the apex of this beak. The centriole begins to enlarge into a rod-shaped structure following division I. It functions as a typical centriole in division II. During the following interkineses, the process of enlargement in contact with the centromeres continues. It again functions as a typical centriole during the third mitosis. (For illustrations, see Plates I and II, Dodge, 1927). Following the third division, the greatly elongated centriole, associated with the beak of each nucleus, comes to lie close to the ascus wall. Fibers emerge from it and encompass a mass of cytoplasm about each nucleus thus initiating spore wall formation. That centromeres, centrioles and blepharoplasts are interchangeable cell organelles has been demonstrated in the classic investigations of Pollister and Pollister (1948). In line with these investigations, it is possible to consider that the centromeres of *Neurospora* may contribute to the substance of the centriole during these periods of enlargement. Centromeres, centrioles and blepharoplasts all have the common function of producing fibers. It is possible that the fibers formed by these three interrelated but morphologically distinct cellular organelles are structurally identical or much alike in that they all possess one particular type of molecular organization which is responsible for their capacity to contract or alternately contract and expand.

SUMMARY

A summary report is given of the results obtained from a very brief study of chromosome and nuclear behavior in *Neurospora crassa*. The investigations are admittedly incomplete and possibly some errors have been made. Nevertheless, they have revealed that *Neurospora* offers adequate and in some respects unique opportunities for cytogenetic research. The chromosomes were followed from the nuclear division preceding zygote formation through the division in the ascospore. Chromosome morphology was considered with reference to the absolute and relative sizes of the seven chromosomes in various division cycles, the centromere positions, the nucleolus chromosomes, the pachytene chromomere morphology and the presence of heterochromatin. Chromosome behavior was followed with reference to the atypical timing of chromosome synapsis, the elongation of the chromosomes during a prolonged "pachytene," chiasma formation and the general behavior of the chromosomes in the two meiotic mitoses and the two subsequent equational mitoses. Several re-

ciprocal translocations were investigated and their usefulness for special studies indicated.

DEPARTMENT OF GENETICS,
CARNEGIE INSTITUTION OF WASHINGTON,
COLD SPRING HARBOR, NEW YORK

LITERATURE CITED

- COLSON, B. 1934. The cytology and morphology of *Neurospora tetrasperma* (Shear and Dodge). *Ann. Bot.* 48: 211-224.
- DODGE, B. O. 1927. Nuclear phenomena associated with heterothallism and homothallism in the ascomycete *Neurospora*. *Jour. Agric. Res.* 34: 289-305.
- HARPER, R. A. 1905. Sexual reproduction and the organization of the nucleus in certain mildews. *Carnegie Inst. Wash. Pub.* 37: 104 p.
- LINDERGREN, C. C., AND S. RUMANN. 1938. The chromosomes of *Neurospora crassa*. *Jour. Genetics* 36: 395-404.
- POLLISTER, A. W., AND P. F. POLLISTER. 1943. The relation between centriole and centromere in atypical spermatogenesis of viviparid snails. *Ann. New York Acad. Sci.* 45: 1-48.
- WILCOX, M. S. 1928. The sexuality and arrangement of the spores in the ascus of *Neurospora sitophila*. *Mycologia* 20: 3-17.

NEUROSPORA. II. METHODS OF PRODUCING AND DETECTING MUTATIONS CONCERNED WITH NUTRITIONAL REQUIREMENTS¹

G. W. Beadle and E. L. Tatum

THE GENERAL procedure by which mutant types of *Neurospora* with altered growth factor requirements are produced and detected has been described several times (Beadle and Tatum, 1941; Tatum, 1944; Horowitz *et al.*, 1945; Beadle, 1945). The purpose of this paper is to give in detail the methods used and the results obtained. Briefly reviewed, the method is as follows: Mutations are induced by ultraviolet treatment, x-radiation or neutron bombardment. Genetically homogeneous strains are then established on a medium containing known vitamins, amino acids and other compounds of suspected biological importance. Loss of ability to carry out the synthetic processes characteristic of the original strain is detected by transferring these pure lines to a "minimal" medium on which growth depends on the ability of the organism to carry out all the major biosyntheses of which the original strain is capable. The heterothallic species of *Neurospora*

are well suited to this type of investigation because their synthetic abilities are well developed, because they can be grown readily in pure culture on a chemically defined medium, and because their life cycle makes genetic control and analysis simple (Shear and Dodge, 1927; Dodge, 1927; Lindegren, 1942).

MATERIALS AND METHODS.—*Stocks.*—Wild-type strains of *Neurospora sitophila* and of *N. crassa* were obtained from Dr. B. O. Dodge, Prof. C. C. Lindegren, Dr. W. S. Malloch, Prof. St. John P. Chilton and Dr. E. V. Abbott. Following a suggestion of Doctor Dodge, we designate the two sexes or mating types *A* and *a*, corresponding respectively to "+" and "—" in Lindegren's terminology. The following stocks have been used:

SA—*N. sitophila* derived from material collected in Bermuda and sent to us by Dodge.

Sa—*N. sitophila*, as above, opposite sex.

LA—*N. crassa* from Lindegren via Malloch.

La—*N. crassa*, as above, opposite sex.

1A—*N. crassa*, single ascospore strain derived from cross of LA and La.

10a—*N. crassa*, as above, opposite sex.

19a—*N. crassa*, as above.

25a—*N. crassa*, as above.

Abb 4A—*N. crassa*, single ascospore strain from material collected by Abbott in Louisiana.

Abb 12a—*N. crassa*, as above, opposite sex.

Ch-a—*N. crassa*, from material collected by Chilton in Louisiana.

Culture media.—As shown by Butler, Robbins and Dodge (1941) and by Beadle and Tatum (1941) wild-type strains of *Neurospora* require the vitamin biotin. If supplied this, they grow on a medium containing a carbon and energy source, an inorganic nitrogen source, plus several inorganic salts. The addition of asparagine or aspartic acid is stimulatory (Bonner, unpublished observations). The basic medium we have used, often referred to as "minimal" medium, contains the following in grams per liter of solution: ammonium tartrate 5,

¹ Received for publication August 28, 1945.

The studies reported here were aided by grants from The Rockefeller Foundation, from the Penrose Fund of the American Philosophical Society, and from The Williams-Waterman Fund of the Research Corporation. Essential stocks were obtained through the kindness of Dr. B. O. Dodge of the New York Botanical Garden, Prof. C. C. Lindegren of Washington University, Dr. W. S. Malloch of the University of California, Dr. E. V. Abbott of the United States Department of Agriculture at Houma, Louisiana, and Prof. St. John P. Chilton of Louisiana State University. Dr. Brewer Boardman and Dr. David B. Nicodemus of the Stanford Department of Physics assisted in the treatment of material with x-rays. Dr. Alfred Marshak and Dr. Muriel V. Bradley of the University of California arranged for and assisted with neutron treatments. For invaluable assistance in the tedious task of establishing single ascospore cultures, testing them for ability to carry out specific syntheses and for maintaining stocks, gratitude is expressed to the following persons: Miss B. Helen Berman, Miss Louise C. Brown, Miss Helen R. Buss, Miss Hyla A. Cook, Miss Verna L. Coonradt, Miss Viola M. Coonradt, Miss Esther H. Davis, Miss J. Hermione Grant, Dr. Russell P. Hager, Mr. Francis T. Haxo, Mrs. Mary B. Houlahan, Mr. L. Louis Howell, Mrs. Mary V. G. Hungate, Mr. Robert N. McFadden, Mr. Robert F. Merchant, Mrs. Caryl Moerdyke, Mrs. Elizabeth S. Pierce, Miss Gerda Straus, Miss Janet M. S. Wallace and Miss A. Jeanne Vincenti.

NH_4NO_3 1, KH_2PO_4 1, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.5, NaCl 0.1, CaCl_2 0.1, sucrose 15, biotin 5×10^{-6} . The following trace elements, added as salts, are present as follows in milligrams per liter of medium: Bo 0.01, Cu 0.1, Fe 0.2, Mn 0.02, Mo 0.02, Zn 2.0. Whenever possible minimal medium is used in liquid form in test tubes or Erlenmeyer flasks. It may be made semi-solid with agar although in this case care must be taken that unwanted extraneous materials are not added with the agar (Robbins, 1939; Ryan, Beadle and Tatum, 1943). This medium may be modified in various ways. Its pH as normally made up is 5.6. In special tests for detecting mutants sensitive to changes in hydrogen ion concentration, we have lowered the pH to 4.6 with HCl or raised it to 7.0 with Na_2HPO_4 . Cellobiose, maltose, trehalose, soluble starch and cottonseed oil emulsion have been substituted for sucrose in tests for ability of strains to utilize specific carbon sources. The NH_4 ions have been replaced by K in tests for ability of strains to utilize NO_3 -nitrogen.

Various so-called complete media have been used from time to time. The one in current use is made up of the following: Fries 3 solution (minimal medium without sucrose or biotin) 1 l., agar 15 g., Difco yeast extract 2.5 g., spray-dried malt syrup 5 g., sucrose 5 g., glucose 5 g., vitamin solution 10 ml., and hydrolyzed casein 5 ml. The vitamin solution contains the following in milligrams per liter of solution: thiamin 100, riboflavin 50, pyridoxin 50, pantothenic acid 200, *p*-aminobenzoic acid 50, nicotinamide 200, choline 200, inositol 400, alkali-hydrolyzed yeast nucleic acid 500, and folic acid equivalent to 4 μg . pure substance. The casein hydrolyzate is prepared by HCl hydrolysis and is made up to the equivalent of 50 mg. casein per ml.

For stock cultures a medium is used similar to the above with two per cent glycerol replacing the sucrose and glucose. Considerable variability has been observed in the sporulation of vegetative cultures of mutant types on different media. In an attempt to find a medium that would be favorable for as many mutant types as possible, Miss Hermione Grant has investigated the growth and sporulation of two wild-type strains, two morphological variants, and twelve biochemical mutants on twelve different media. The media included variations in trace elements, substitution of glucose and sucrose for glycerol, replacement of agar with "carragar," an agar substitute manufactured from *Chondrus crispus*, and the use of whole yeast instead of yeast extract. In addition to these, the carrot-beet-cucumber-potato-extract medium of Mrak *et al.* (1942), a hay-infusion-peptone-agar medium, and a medium in which glycerol, molasses, peptone, and five per cent NaCl are the principal ingredients were used. Unfortunately it is found that no one medium is most favorable for all strains. The glycerol, yeast extract, malt syrup, vitamin, casein, and agar medium mentioned above is as good an all-purpose stock culture medium for *Neurospora* as we have found. For individual mutant strains it is often

necessary to devise special media. For example, Doermann (1944) has shown that lysineless mutants are specifically inhibited if the molar concentration of arginine in the culture medium exceeds that of lysine. Often minimal medium agar supplemented with the specific substance required by a particular strain is satisfactory (Regnery, 1944).

A so-called coconut medium has been used which is designed to provide heat-labile substances that might be required by mutant strains. The use of coconut for this purpose was suggested to us by the work of Overbeek *et al.* (1942, 1944). Coconut medium is made by grinding the meat of a ripe coconut in a food chopper, adding 200 ml. of distilled water, homogenizing in a Waring blender for five to ten minutes and then straining through two layers of cheese cloth. The resulting cream-like material is added to Fries 3 solution in ten per cent concentration. In addition agar (3%), sucrose (1%), yeast extract (0.1%) and dried malt syrup (0.1%) are added. This medium is distributed to culture tubes in amounts equal to half the desired final quantities of medium. Following autoclave sterilization and cooling to about 50°C., equal quantities of the aseptic liquid of a half-ripe coconut are added to the culture tubes. The agar medium and coconut "milk" are mixed and allowed to cool for use. The coconut milk is removed aseptically with a large syringe and suitable sized hypodermic needle and is distributed to culture tubes directly from this syringe. The various complete media described are not favorable for cultures in which fertile perithecia are desired. For this purpose we use Difco cornmeal agar, an agar medium containing an infusion of cornmeal and 0.2 per cent glucose.

INDUCTION OF MUTATIONS.—Three types of treatment were used for the induction of mutations, x-rays, ultraviolet radiation and neutrons. The x-ray apparatus used is of a special design described briefly by Taylor, Thomas and Brown (1933). A silver target was used and the tube operated at 40 KV. and 10 ma. The spores being rayed were placed 4 cm. from the target. The radiation was unfiltered except by the aluminum window of the tube. This window is 0.002 inch in thickness. Under these conditions, as shown by calibration with a Victoreen meter, x-rays were delivered at the rate of 550 r units per second. Material at two stages in the life cycle was rayed. In earlier experiments, young perithecia, taken prior to meiosis, were rayed. These were allowed to complete development and subsequent to this, one ripe ascospore was taken from each perithecium. Strains derived from these were then investigated to determine their nutritional requirements. In later treatments, dry conidia were rayed and then applied to protoperithecia of the opposite sex. Dodge (1932) and Backus (1939) have shown that the nuclei of conidia applied to protoperithecia in this way are taken into the perithecia and contribute to the formation of zygote nuclei. In this case too, only one ascospore per mature perithecia was taken for the establishment

of lines to be tested for change in nutritional requirements. In both methods of treatment the taking of a single ascospore per perithecium insures that only one ascospore will be taken carrying descendants of a particular mutant gene produced by the treatment. Thus if two mutants concerned with the same biosynthetic reaction are found, they are presumed to be of independent origin. There is some possibility of inadvertently taking two spores from a single perithecium. To make possible the elimination of any such errors, the cultures from the ascospores of a single tube of fertile perithecia were distinguished from those from independent tubes. Thus rayed conidia were applied to protoperithecia in several culture tubes. From each of these, ascospores were taken, one from each mature perithecium, and identified as to which culture tube they came from. If identical mutant strains came from a single tube, they may conceivably carry descendants of a single original mutant gene. If, on the other hand, they come from separate tubes, they almost certainly represent independent occurrences of the same mutation.

Ultraviolet treatments were made in essentially the same way. A dilute suspension of conidia of a wild-type strain was distributed in small Syracuse dishes, these placed under the tube of a Westinghouse "Sterilamp" and irradiated for the desired time with constant agitation of the suspension. The type of lamp used emits approximately 85 per cent of its energy at 2537 Å. Dosages were computed after calibration of the lamp with a Hanovia photocell ultraviolet meter. At a distance of two inches from the tube the incident ultraviolet energy so computed was 5,500 ergs/mm²/min.

Neutron treatments were made with the help of members of the Radiation Laboratory of the University of California, using the 60 inch cyclotron. Dosages were calculated from ionization measurements at the collimator port and are expressed in n-units.

Since our purpose was to obtain mutations of specific types, not to determine their frequency in relation to dosage, wave length or other variables, we have not taken the precautions necessary to obtain measurements of dosages and mutation rates of the highest accuracy possible. Thus the x-ray tube and the ultraviolet lamp were not calibrated at every treatment and the line voltage on the ultraviolet lamp was not kept constant. The neutron treatments were preliminary and the dosages are very rough approximations.

The procedure outlined above was followed in preference to the method of treating microconidia used by Lindegren and Lindegren (1941a, 1941b), Hollaender *et al.* (1945) and by Sansome *et al.* (1945) because it makes certain that all nuclei of a given strain are genetically alike, barring spontaneous mutation. With the method of treating microconidia there is a possibility of obtaining heterocaryons that make subsequent analysis difficult if

any spores are binucleate or if the chromosomes are effectively double at the time of treatment.

Spore isolations.—Our method of making ascospore isolations involves the use of blocks of four per cent plain agar about $\frac{1}{4} \times \frac{3}{4} \times 2$ inches. These are cut from poured petri plates, placed on standard microscope slides and worked on under a dissecting microscope at a magnification of 10 to 48 diameters. With a platinum-iridium transfer needle with a lance-like tip, single ascospores are transferred to the edge of the agar block. They are lined up in a row about one mm. from the edge and about two mm. apart on the row. With the transfer lance blocks of agar are cut about two mm. on a side and a half a mm. thick, each block having a spore on its top side. These blocks are then picked up on the transfer lance and placed spore-side-up on the surface of the medium in a culture tube. Small culture tubes $3 \times \frac{3}{8}$ inches are convenient for such single spore cultures. Ascospores are heat-activated (Shear and Dodge, 1927; Goddard, 1939) by placing racks of culture tubes in a 60°C. water bath for 30 minutes.

In isolating ascospores in order, a somewhat similar procedure is used. Perithecia are squeezed with fine forceps in such a way as to crack them. The clusters of asci so obtained are spread on the agar block. With a glass micro-needle operated free-hand, the asci are arranged so that the spores from a single one can be removed through a distal rupture in the ascus. The eight individual spores are lined up in order along the edge of the agar block. At this stage they are flooded with a 1.5 per cent sodium hypochlorite solution (50 per cent commercial "Clorox," "Purex" or equivalent hypochlorite solutions are suitable). With the transfer lance a drop of the solution is drawn over the row of eight spores. They are then cut out on individual blocks and transferred to culture tubes. The hypochlorite treatment kills conidia, mycelial fragments and most contaminants and permits the aging of ascospores for several days prior to heat-activation. Such aging often greatly increases the percentage germination.

Tests for mutations.—Strains from ascospores carrying mutant alleles of genes necessary for the synthesis of vitamins, amino acids and other biologically active compounds are able to survive on complete medium because the substances they cannot synthesize are supplied in the medium. Such strains are separated from those that are not modified in their growth factor requirements by subculturing all strains on one or more minimal media. The minimal medium we have made most extensive use of is the one containing sucrose as a carbon source. To grow on it a strain must of course synthesize all necessary vitamins, amino acids and other compounds necessary for growth, except biotin which is supplied in the medium. Growth on complete medium and lack of it on sucrose minimal medium indicates either failure to carry out the synthesis of some substance present in complete and

TABLE 1. Summary of tests for mutations induced by x-ray treatment.

Dosage r-units	Medium with pH and temperature at which used										Cottonseed oil				Biochemical mutants				
	Sucrose 5.6 25°		Sucrose 4.6 25°		Starch 5.6 25°		Starch—NO ₃ 7.0 33°		Maltose 5.6 25°		Cellobiose 5.6 25°		Trehalose 5.6 25°		Sucrose—NO ₃ 5.6 25°		Good		Ques- tionable
	586	606	586	606	586	606	586	606	586	606	586	606	586	606	586	606	586	606	
3,300 ^{ab}	586	606	586	606	586	606	586	606	586	606	0
3,300 ^a	606	2,499	606	2,499	606	1,169	606	1,124	606	1,124	1
4,400 ^a	2,499	1,773	1,149	349	2,499	630	1,169	1,124	...	3
8,250 ^a	1,773	3,932	349	1,533	630	11
11,000 ^a	3,932	1,533	21
11,000	1,261	887	0
22,000	6,851	311	948	4,392	2,644	17	31
27,500	...	3,243	3,242	5	1
33,000	2,210	2,210	3	5
44,000	4,559	2,450	4,427	950	10	17
55,000	2,388	1,815	3	6
Total	26,665	3,243	4,223	5,223	3,242	6,809	2,361	8,819	3,594	2,316	72	96							

^a Immature perithecia rayed, i.e., both parental sets of chromosomes treated.^b *Neurospora sitophila*.

not present in minimal or failure to utilize sucrose as a carbon source. Other minimal media have been used. By observing growth responses on these, it is possible to detect mutant types with altered pH sensitivities, those unable to grow throughout the temperature range at which wild-type strains will grow, strains unable to utilize specific substances as carbon sources, and those unable to utilize nitrate nitrogen.

Strains that show growth on complete medium but not on various minimal media are investigated further by sub-culturing them on sucrose minimal medium supplemented with (1) the vitamin mixture described above (1 ml. vitamin mixture per 100 ml. medium), (2) casein hydrolysate (1 ml. per 100 ml. medium), (3) glucose minimal, and (4) a complete medium control. Growth on media (1) and (4) but not on (2) and (3) indicates failure of the strain to synthesize sufficient quantities of one or more of the vitamins in the mixture. Which of the vitamins in the mixture is required by such altered strains is then determined by sub-culturing on media supplemented by individual vitamins. Growth on media (2) and (4) but not (1) and (3) indicates that one or more amino acids are required for growth. The specific requirement is established by observing sub-cultures on minimal media supplemented by individual amino acids or combinations of these. A third category of mutant types is indicated by growth on complete but failure to grow on either (1), (2) or (3). Strains falling into this category may have lost the ability to synthesize some one or more compounds of biological importance not present in either the vitamin mixture or the casein hydrolysate. There are, however, other possibilities; for example, they may be double mutants requiring both a vitamin and an amino acid, or they may have a single requirement but be specifically inhibited by a second substance in the mixture.

It is found that some spores from irradiated material germinate on complete medium but give hyphae which grow very slowly or fail to develop further after growing only a millimeter or so. On the assumption that some of the slow-growing or rudimentary mycelia are unable to synthesize essential heat-labile substances, they were routinely tested for ability to grow on coconut medium.

Folic acid was used in the vitamin mixture at a concentration just sufficient to give growth of *Streptococcus lactis* R. If the requirement of a *Neurospora* strain unable to make folic acid were appreciably higher than this, it is possible that such strains would be missed on tests on the vitamin mixture. Either this or breakdown of the folic acid in our stock solution (kept under toluene in the refrigerator) may account for the apparent lack of mutant types requiring folic acid for growth.

RESULTS.—The observed frequencies of so-called biochemical mutants in different experiments are given in tables 1, 2 and 3. Mutants are classified as "good" and "questionable." It is recognized that

TABLE 2. Summary of tests for mutations induced by ultraviolet radiation.

Dosage ergs/mm ²	Medium with pH and temperature at which used							Biochemical mutants	
	Sucrose 5.6 25°	Sucrose 4.6 25°	Starch— NO ₃ 5.6 25°	Starch— NO ₃ 7.0 25°	Starch— NO ₃ 7.0 33°	Cellobiose 7.0 33°	Trehalose 7.0 33°	Good	Ques- tionable
9,400	2,415	2,415	11	14
9,400	2,239	9,048	8,676	8,676	100	47
9,400	245	246	246	2	2
9,400	9,373	6,125	3,106	3,613	2,439	101	23
9,400	1,373	1,369	7	4
9,400	4,197	4,187	34	0
11,750	4,551	4,367	32	14
11,750	2,304	2,141	21	4
14,100	1,142	938	0	3
18,800	128	128	0	0
Total	10,475	26,540	7,848	15,047	10,803	12,535	2,439	308	111

this classification is by no means entirely objective. The data are given mainly to indicate orders of magnitude.

Several of the biochemical mutants tabulated are types that show little or no growth on regular complete medium but grow normally or nearly so on coconut medium. The factor responsible for improved growth on coconut medium has not been determined in any instance.

In addition to the "biochemical" mutants, a number of "morphological" mutants were observed and recorded. Although many of these have been saved, no record of their frequency is given because of the large subjective factor in their detection and its variability from one worker to another.

TABLE 3. Summary of tests for mutations induced by neutrons.

Dosage n-units	Sucrose 4.6 25°	Starch—NO ₃ 7.0 33°	Biochemical mutations	
			Good	Ques- tionable
2,000	312	312	0	0
3,400	963	963	2	0

Many instances have been recorded in which spores show germination but in which growth of hyphae is very slow or does not take place on either regular complete medium or on coconut medium. No doubt, many such spores carry mutant genes of such a nature that the mycelia derived from them require substances either not present in the complete media or not capable of being taken up from these media.

No classification is given of mutant types according to the supplement required for growth because these have not been determined for all mutants and because in many instances where they have been determined further work is necessary to verify the determination. The great majority of the mutant strains obtained have turned out to require vitamins,

amino acids, purines or pyrimidines for normal growth. A few require substances as yet not known to us. Several are unable to utilize nitrate nitrogen, two have been found in which fat is not utilized normally as a carbon and energy source (Horowitz *et al.*, 1945). More than a dozen "temperature-sensitive" mutants have been identified. They grow normally on minimal medium at one temperature within the normal range for wild-type, but not at another. An example of a mutant of this type is described by Mitchell and Houlahan (*in press*). No clear case of a mutant unable to utilize a specific sugar has been found nor has any strain been found which cannot grow on starch as a carbon and energy source.

Examination of the data summarized in table 1 gives evidence of an increasing mutation rate with increasing x-ray dosage in material rayed as young perithecia. When conidia were treated, on the other hand, there is no convincing evidence of a simple relation between mutation rate and dosage. While there appears to be no reason for suspecting any error in the calculation of dosages, at least not in relative values since only the time factor was varied in different treatments, more satisfactory quantitative data on dosage and frequency of biochemical mutations are needed before any serious attempt at explanation of the situation is justified.

For purposes of determining from which stocks and treatment any mutant strain referred to here or elsewhere came, an index of all culture numbers is given in table 4.

Genetic investigations.—Whether or not a metabolic change is due to a gene modification can be determined only by the methods of classical genetics. Crosses between mutant strains and the wild-type from which they were derived were routinely made. These are made on cornmeal agar either by "conidiating" wild-type protoperithecia with conidia or mycelial fragments of the mutant strain (Dodge, 1932) or by allowing wild-type and mutant mycelia to grow together on the surface of the medium. Studies of the inheritance of the changes

TABLE 4. Index of culture numbers. (In series in which dosage is followed by an asterisk young perithecia were rayed. In all other instances conidia were rayed.)

Number beginning sequence	Strains	X-ray dosage (r-units)	Ultraviolet dosage (ergs/mm ²)	Number beginning sequence	Strains	X-ray dosage (r-units)	Ultraviolet dosage (ergs/mm ²)
1	SA × Sa	3,000*	28,041	1A × 19a	33,000	
308	LA × La	3,300*	28,067	1A × 19a		9,400
953	SA × Sa	3,300*	28,098	1A × 19a	33,000	
1,274	LA × La	4,400*	28,133	1A × 19a		9,400
3,933	LA × La	8,250*	28,166	1A × 19a	33,000	
5,451	LA × La	11,000*	28,317	1A × 19a		9,400
9,241	LA × La	11,000	28,361	1A × 19a	33,000	
9,301	LA × La	8,252*	28,406	1A × 19a		9,400
9,741	LA × La	11,000	28,409	1A × 19a	33,000	
9,801	LA × La	11,000*	28,454	1A × 19a		9,400
10,429	LA × La	11,000	28,505	1A × 19a	33,000	
11,138	LA × La	22,000	28,550	1A × 19a		9,400
11,424	LA × La	11,000	28,703	1A × 19a	33,000	
11,621	LA × La	22,000	28,712	1A × 19a		9,400
12,033	LA × La	11,000	28,826	1A × 19a	33,000	
12,285	LA × La	22,000	28,895	1A × 19a		9,400
12,874	LA × La	44,000	28,984	1A × 19a	33,000	
12,986	LA × La	22,000	29,009	1A × 19a		9,400
13,089	LA × La	44,000	29,046	1A × 19a	33,000	
13,127	LA × La	22,000	29,099	1A × 19a		9,400
13,193	LA × La	44,000	29,155	1A × 19a	33,000	
13,257	LA × La	22,000	29,167	1A × 19a		9,400
13,511	LA × La	44,000	29,225	1A × 19a	33,000	
13,569	LA × La	22,000	29,331	1A × 19a		9,400
14,287	LA × La	44,000	29,397	1A × 19a	33,000	
14,347	LA × La	22,000	29,533	1A × 19a		9,400
14,782	LA × La	44,000	29,572	1A × 19a	33,000	
15,218	LA × La	22,000	29,631	1A × 19a		9,400
15,435	LA × La	44,000	29,682	1A × 19a	33,000	
15,466	LA × La	22,000	29,928	1A × 19a		9,400
15,662	LA × La	44,000	30,166	1A × 19a	33,000	
16,118	La × LA	22,000	30,190	1A × 19a		9,400
16,884	La × LA	44,000	30,776	1A × 19a	33,000	
16,873	La × LA	22,000	30,821	1A × 19a		9,400
20,951	LA × La	44,000	31,532	1A × 19a	33,000	
24,428	La × LA	55,000	32,006	1A × 19a		11,750
24,916	LA × La	55,000	34,559	1A × 19a		14,100
25,906	La × LA	55,000	34,564	1A × 19a		18,800
26,586	19a × 1A	55,000	35,001	1A × 25a		9,400
26,593	1A × 19a	55,000	40,101	Abb4 × Abb12		9,400
26,819	1A × 19a	11,750	44,001	Abb4 × 25a		9,400
26,958	1A × 19a	33,000		49,001	Abb4 × Ch a		9,400
27,430	1A × 19a		11,750	55,001	Abb4 × Ch a	27,500	
27,520	1A × 19a	33,000		60,501	Abb12 × Abb4		9,400
27,651	1A × 19a		11,750	63,001	Abb4 × 25a		9,400
27,699	1A × 19a	33,000		67,501	Abb4 × 25a		11,750
27,750	1A × 19a		11,750	75,001	Abb4 × 25a	(neutrons 3,400 n-units)	
27,792	1A × 19a	33,000		78,701	Abb4 × 25a	(neutrons 2,000 n-units)	
27,986	1A × 19a		9,400				

induced in the more than 500 strains indicated in tables 1, 2 and 3 has by no means been completed. However, a fair sample of the mutant strains has been studied in this way and in the majority of them the "mutations" are found to involve heritable alterations that are transmitted as single gene changes. Occasionally double mutations have been found, but the incidence of these is apparently no greater than might be expected on the assumption that the two mutations were independently induced. Table 3

summarizes the meager data on neutron-induced mutations. The two mutations obtained constitute too limited a sample on which to base significant conclusions as to the frequency or kinds of mutant types produced by such treatment.

A list of those mutants to which reference has already been made in the literature or is made in papers of this series is given in table 5. In all instances listed, genetic investigation has shown that the mutant strain differs from the wild-type

TABLE 5. Summary of mutant strains of *Neurospora* that have been referred to in the literature. (All strains are *N. crassa* except those marked with an asterisk, which are *N. sitophila*. Strains known to represent recurrences of listed mutant types are indicated by an R following the culture number.)

Substance required for growth	Culture number	Treatment	References
Thiamin	1,090*	X-ray	Beadle and Tatum, 1941; Tatum and Bell, 1945
	9,185	X-ray	Tatum and Bell, 1945
	17,084	X-ray	Tatum and Bell, 1945
	50,005	Ultraviolet	Houlahan and Mitchell, unpublished
	56,501	X-ray	Tatum and Bell, 1945
Thiazole	18,558	X-ray	Tatum and Bell, 1945
Riboflavin	51,602	Ultraviolet	Mitchell and Houlahan, 1945
Pyridoxin	299*	X-ray	Beadle and Tatum, 1941; Stokes <i>et al.</i> , 1943
Nicotinic acid	3,416	X-ray	Beadle and Coonradt, 1944; Bonner and Beadle, unpublished
	4,540	X-ray	Beadle and Coonradt, 1944; Bonner and Beadle, unpublished
	39,401	Ultraviolet	Bonner and Beadle, unpublished
Pantothenic acid	5,531	X-ray	Tatum, 1944
	34,556R	Ultraviolet	This paper
p-Aminobenzoic acid	1,633	X-ray	Tatum and Beadle, 1942; Thompson <i>et al.</i> , 1943
Inositol	37,101	Ultraviolet	Beadle, 1944
	37,401R	Ultraviolet	Beadle, 1944
	46,316R	Ultraviolet	Beadle, 1944
	46,802R	Ultraviolet	Beadle, 1944
	64,001R	Ultraviolet	Beadle, 1944
Choline	34,486	Ultraviolet	Horowitz and Beadle, 1943; Horowitz <i>et al.</i> , 1945
	34,542R	Ultraviolet	This paper
	37,903R	Ultraviolet	This paper
	47,904	Ultraviolet	Horowitz <i>et al.</i> , 1945
	66,210R	Ultraviolet	This paper
Ornithine	21,502	X-ray	Srb and Horowitz, 1944
	27,947	X-ray	Srb and Horowitz, 1944
	29,997	Ultraviolet	Srb and Horowitz, 1944
	34,105	Ultraviolet	Srb and Horowitz, 1944
Citrulline	30,330	Ultraviolet	Srb and Horowitz, 1944
	33,442	Ultraviolet	Srb and Horowitz, 1944
	36,703	Ultraviolet	Srb and Horowitz, 1944
Arginine	16,117	X-ray	Bonner <i>et al.</i> , 1943
Isoleucine, valine	33,757	Ultraviolet	Regnery, 1944; Ryan and Brand, 1944
Leucine	4,545	X-ray	Doermann, 1944
Lysine	4,894	X-ray	Horowitz <i>et al.</i> , 1945; Buss, 1944
Methionine	21,863	X-ray	Horowitz <i>et al.</i> , 1945; Bonner, unpublished
Proline	40,008	Ultraviolet	Tatum <i>et al.</i> , 1944; Tatum and Bonner, 1944
Anthranilic acid	10,575	X-ray	Tatum <i>et al.</i> , 1944; Tatum and Bonner, 1944
Indole	33,050	Ultraviolet	Horowitz <i>et al.</i> , 1945; this paper
Valine	3,254	X-ray	Pierce and Loring, in press
Adenine	H263 ^a	X-ray	Loring and Pierce, 1944
Cytidylic acid	1,298	X-ray	Loring and Pierce, 1944
	45,203R	Ultraviolet	Loring and Pierce, 1944
Nitrite or other reduced nitrogen	14,789	X-ray	Horowitz <i>et al.</i> , 1945

^a Obtained by Mr. Frank P. Hungate.

strain from which it came in a manner consistent with the assumption that the mutational change involved a single gene.

One naturally wonders what proportion, if any, of the alterations involves physical deficiencies. Since in the majority of cases the mutant strains become essentially normal when supplied with a single additional substance in the medium, it seems probable that if deficiencies are involved they are single gene deficiencies or very small ones involving the loss of only one gene with major effects. One reason for obtaining mutant strains of this type is

to find out what genes do. This can be determined either by inactivating genes or by destroying them. In either case we are able to deduce something as to the function of the unaltered gene. It is possible to rule out destruction if it can be shown that back mutation to the normal allele is possible. We have not as yet made extensive studies of this nature. In preliminary studies carried out in collaboration with the late Prof. Leon H. Leonian of the University of West Virginia we have obtained evidence that the mutant gene differentiating 1633 (amino-

benzoicless) from wild-type is capable of back mutation.

Given sufficient data of the type listed in table 5, it should be possible to determine whether the relative susceptibilities of different genes to x-rays is the same as that to ultraviolet radiation. Unfortunately our data are not yet sufficient to give a final answer to this. There appear to be some rather curious differences, for example, the fact that all but one of the mutants having to do with thiamin were produced by x-ray treatment, while all those interfering with inositol and choline synthesis were modified by ultraviolet treatment. With the numbers involved, however, these may represent nothing more than chance distributions.

DISCUSSION.—The thesis that genes act by determining the specificities of enzyme proteins and as a consequence control specific chemical reaction has been developed and defended several times (Beadle and Tatum, 1941; Tatum, 1944; Horowitz *et al.*, 1945; Beadle, 1945). The experimental results presented in this paper make up the evidence supporting this as far as control of specific reactions in *Neurospora* is concerned. It is certainly true that if the appropriate experimental conditions can be set up for producing and detecting them, the probability is high of obtaining mutations concerned with almost any synthetic process. Thus in tests of 68,198 single spore strains of *Neurospora*, the chromosomes of which had been treated with x-rays or

ultraviolet radiation, we have obtained mutations with altered capacities for synthesis of most of the B-vitamins and most of the so-called essential amino acids.

There is a conspicuous absence of mutant types in *Neurospora* in which fat-soluble vitamins are required for growth. These have been searched for consistently, but not found. It seems improbable that they were systematically missed. It is possible that these vitamins cannot be utilized when supplied in the medium. On the other hand, it is conceivable that they have no function essential for growth in plants.

SUMMARY

Among 68,198 single-spore strains of *Neurospora crassa* and *N. sitophila* derived from material treated with x-rays, ultraviolet radiation, or neutrons, more than 380 strains with altered nutritional requirements have been obtained. On genetic study, many of these prove to differ by single genes from the wild-type strains from which they were obtained. Most of them have altered requirements for B-vitamins, amino acids, or purine and pyrimidine bases. The relation of such mutant types to the hypothesis that genes in general function in determining enzyme specificities and hence control particular chemical reactions is briefly discussed.

SCHOOL OF BIOLOGICAL SCIENCES,
STANFORD UNIVERSITY, CALIFORNIA

LITERATURE CITED

- BACKUS, M. D. 1939. The mechanics of conidial fertilization in *Neurospora sitophila*. Bull. Torrey Bot. Club 66: 63-76.
- BEADLE, G. W. 1944. An inositolless mutant strain of *Neurospora* and its use in bioassays. Jour. Biol. Chem. 156: 683-689.
- . 1945. Genetics and metabolism in *Neurospora*. Physiol. Rev. 25: 643-663.
- , AND V. COONRADT. 1944. Heterocaryosis in *Neurospora crassa*. Genetics 29: 291-308.
- , AND E. L. TATUM. 1941. Genetic control of biochemical reactions in *Neurospora*. Proc. Natl. Acad. Sci. U.S.A. 27: 499-506.
- BONNER, DAVID, E. L. TATUM, AND G. W. BEADLE. 1943. The genetic control of biochemical reactions in *Neurospora*: a mutant strain requiring isoleucine and valine. Arch. Biochem. 3: 71-91.
- BUSS, HELEN R. 1944. The genetics of methionineless mutants of *Neurospora crassa*. M. A. Thesis, Stanford Univ.
- BUTLER, E. T., W. J. ROBBINS, AND B. O. DODGE. 1941. Biotin and the growth of *Neurospora*. Science 94: 262-263.
- DODGE, B. O. 1927. Nuclear phenomena associated with heterothallism and homothallism in the ascomycete *Neurospora*. Jour. Agric. Res. 35: 289-305.
- . 1932. The non-sexual and sexual functions of microconidia of *Neurospora*. Bull. Torrey Bot. Club 59: 347-360.
- DOERMANN, A. H. 1944. A lysineless mutant of *Neurospora* and its inhibition by arginine. Arch. Biochem. 5: 373-384.
- GODDARD, DAVID R. 1939. The reversible heat activation of respiration in *Neurospora*. Cold Spring Harbor Symposia on Quantitative Biology 7: 362-376.
- HOLLAENDER, A., E. R. SANSOME, E. ZIMMER, AND M. DEMEREC. 1945. Quantitative irradiation experiments with *Neurospora crassa*. II. Ultraviolet irradiation. Amer. Jour. Bot. 32: 226-235.
- HOROWITZ, N. H., AND G. W. BEADLE. 1943. A microbiological method for the determination of choline by use of a mutant of *Neurospora*. Jour. Biol. Chem. 150: 325-333.
- , DAVID BONNER, AND M. B. HOULAHAN. 1945. The utilization of choline analogues by cholineless mutants of *Neurospora*. Jour. Biol. Chem. 159: 145-151.
- , ———, H. K. MITCHELL, E. L. TATUM, AND G. W. BEADLE. 1945. Genic control of biochemical reactions in *Neurospora*. Amer. Nat. 79: 304-317.
- LINDEGREN, C. C. 1942. The use of fungi in modern genetical analysis. Iowa State Coll. Jour. Sci. 16: 271-290.
- , AND G. LINDEGREN. 1941a. X-ray and ultra-violet induced mutations in *Neurospora*. I. X-ray mutations. Jour. Heredity 32: 405-412.
- , ———. 1941b. X-ray and ultra-violet induced mutations in *Neurospora*. II. Ultra-violet mutations. Amer. Jour. Bot. 32: 435-440.
- LORING, H. S., AND J. G. PIERCE. 1944. Pyrimidine nucleosides and nucleotides as growth factors for mutant strains of *Neurospora*. Jour. Biol. Chem. 153: 61-69.
- MITCHELL, H. K., AND M. B. HOULAHAN. 1946. *Neurospora*. IV. A temperature-sensitive riboflavinless mutant. Amer. Jour. Bot. (In press.)
- MRAK, E. M., H. J. PHAFF, AND H. C. DOUGLAS. 1942. A

- sporulation stock medium for yeasts and other fungi. *Science* 96: 432.
- OVERBEEK, J. VAN, M. E. CONKLIN, AND A. F. BLAKESLEE. 1942. Cultivation in vitro of small *Datura* embryos. *Amer. Jour. Bot.* 29: 472-477.
- , R. SIU, AND A. J. HAAGEN-SMIT. 1944. Factors affecting the growth of *Datura* embryos in vitro. *Amer. Jour. Bot.* 31: 219-224.
- PIERCE, J. G., AND H. S. LORING. 1945. Growth requirements of a purine-deficient strain of *Neurospora*. *Jour. Biol. Chem.* (In press.)
- REGNERY, D. C. 1944. A leucineless mutant strain of *Neurospora crassa*. *Jour. Biol. Chem.* 154: 151-160.
- ROBBINS, W. J. 1939. Growth substances in agar. *Amer. Jour. Bot.* 26: 772-778.
- RYAN, F. J., G. W. BEADLE, AND E. L. TATUM. 1943. The tube method of measuring the growth rate of *Neurospora*. *Amer. Jour. Bot.* 30: 784-799.
- , AND E. BRAND. 1944. A method for the determination of leucine in protein hydrolysates and in foodstuffs by the use of a *Neurospora* mutant. *Jour. Biol. Chem.* 154: 161-175.
- SANSOME, E. R., M. DEMEREC, AND A. HOLLAENDER. 1945. Quantitative irradiation experiments with *Neurospora crassa*. I. Experiments with x-rays. *Amer. Jour. Bot.* 32: 218-226.
- SHEAR, C. L., AND B. O. DODGE. 1927. Life histories and heterothallism of the red bread-mold fungi of the *Monilia sitophila* group. *Jour. Agric. Res.* 34: 1019-1042.
- SRB, A. M., AND N. H. HOROWITZ. 1944. The ornithine cycle in *Neurospora* and its genetic control. *Jour. Biol. Chem.* 154: 129-139.
- STOKES, J. L., J. W. FOSTER, AND C. R. WOODWARD, JR. 1943. Synthesis of pyridoxin by a "pyridoxinless" x-ray mutant of *Neurospora sitophila*. *Arch. Biochem.* 2: 235-245.
- TATUM, E. L. 1944. Biochemistry of fungi. *Ann. Rev. Biochem.* 13: 667-704.
- , AND G. W. BEADLE. 1942. Genetic control of biochemical reactions in *Neurospora*: an "Aminobenzoicless" mutant. *Proc. Natl. Acad. Sci. U.S.A.* 28: 234-243.
- , AND T. T. BELL. 1946. *Neurospora*. III. Thiamin synthesis. *Amer. Jour. Bot.* (In press.)
- , AND DAVID BONNER. 1944. Indole and serine in the biosynthesis and breakdown of tryptophane. *Proc. Natl. Acad. Sci. U.S.A.* 30: 30-37.
- , AND G. W. BEADLE. 1944. Anthranilic acid and the biosynthesis of indole and tryptophane by *Neurospora*. *Arch. Biochem.* 3: 477-478.
- TAYLOR, C. V., J. O. THOMAS, AND M. G. BROWN. 1933. Studies on protozoa. IV. Lethal effects of x-radiation of a sterile culture medium for *Colpidium campylum*. *Physiol. Zool.* 6: 467-492.
- THOMPSON, R. C., E. R. ISBELL, AND H. K. MITCHELL. 1943. A microbiological assay method for *p*-aminobenzoic acid. *Jour. Biol. Chem.* 148: 281-287.

INDEX TO VOLUME 32

(New names and new combinations are in bold-face type)

- Absorption, of silicon, by rye and sunflower, 539
- ACONITUM nivatum** sp. nov., 286
- ADDICOTT, FREDRICK T. The anatomy of leaf abscission and experimental defoliation in guayule, 250
- Age, relation to light, variety and thermo-periodicity in tomatoes, 469
- Air-conditioned greenhouse, and field culture of tomatoes, comparison between, 643
- Algae, marine, from southern California, 447
- Alkaloid, production in *Nicotiana*: The origin of nornicotine, 416
- Allium cepa* x *A. fistulosum* hybrid and its derivatives, studies on chiasmata of, 370
- AMSONIA **biformis** sp. nov., 288
- Anatomy, developmental, of *Phlox Drummondii* Hook. The embryo, 588; the seedling, 628; of *Cryptostegia grandiflora*, special reference to latex system, 135; of leaf abscission and experimental defoliation in guayule, 250
- ANDRUS, C. F. (See P. H. HEINZE, 62)
- Aneuploid, chromosome numbers, production of, within the root tips of *Paphiopedilum Wardii*, 506
- Apex, shoot, *Sequoia sempervirens* (Lamb.) Endl., growth and vascular development in, 118
- Apomictically, produced plants, in F₁ progenies of guayule (*Parthenium argentatum*) and mariola (*P. incanum*), genetic variation among, 554
- Arenaria ovalifolia** (Hook.) comb. nov., 285
- Asparagus*, cultivation of excised stem tips in vitro, 13; *A. officinalis* L., a cytogenetic study of polyembryony in, 560
- Aspergillus*, cell walls of, in relation to theory of cellulose particles, 148; *A. terreus*, production and characterization of ultraviolet-induced mutations in, 160, 165, 214
- Autotetraploid, barley, colchicine-induced, 103, 177, 180
- Auxin, relationships to nitrogen in green plants, 666; extraction from tomato fruit, 242; inhibitory effect on bud growth in guayule, 270; relation to polyploidy and nitrogen in green plant tissue, 669; rapid total extraction of, from green plant tissue, 188
- Avena*, coleoptiles of different ages, cell number in successive segments of, 575
- AVERY, G. S., JR., J. BERGER, and R. O. WHITE. Rapid total extraction of auxin from green plant tissue, 188; and LOUISE POTTORF. Auxin and nitrogen relationships in green plants, 666; Polyploidy, auxin and nitrogen in green plant tissue, 669; MARGARET PIPER and PATRICIA SMITH. Cell number in successive segments of *Avena* coleoptiles of different ages, 575
- Avicenniaceae, and Verbenaceae, geographic distribution of, 609
- Barley, colchicine-induced autotetraploid, 103, 177, 180
- BEADLE, G. W. and E. L. TATUM. *Neurospora* II. Methods of producing and detecting mutations concerned with nutritional requirements, 678
- Bean, accuracy of the local lesion method for measuring virus activity in, 613
- BERGER, J. (See G. S. AVERY, JR., 188)
- BETULA **beeniana** sp. nov., 284
- BIDDULPH, ORLIN and DONALD H. BROWN. Growth and phosphorus accumulation in cotton flowers as affected by meiosis and fertilization, 182
- Biochemical, characteristics of ultraviolet-induced mutations in *Aspergillus terreus*, 214
- BLACK, L. M. A virus tumor disease of plants, 408
- BLASER, H. WESTON. Anatomy of *Cryptostegia grandiflora* with special reference to the latex system, 135
- BLOCH, ROBERT (See EDMUND W. SINNOTT, 151)
- BOWDEN, WRAY M. A list of chromosome numbers in higher plants. I. Acanthaceae to Myrtaceae, 81; II. Menispermaceae to Verbenaceae, 191
- BRANNON, MELVIN AMOS, and HAROLD MELVIN SELL. The effect of indole-3-acetic acid on the dry weight of *Chlorella pyrenoidosa*, 257
- Brazilian chytrids, observations relative to sexuality in two new species of *Siphonaria*, 580; polycentric species of, 29; *Rhopalophlyctis* and *Chytriomycetes*, two new chitino-phyllid operculate genera of, 362
- Breeding, of ornamental edible peaches, inheritance of tree and flower characters, 53; structure, in *Bromus carinatus*, as determined by population analysis, 142
- Bromus carinatus*, Hook. & Arn., cleistogamy and chasmogamy in, 66; natural breeding structure in, as determined by population analysis, 142
- BROWN, DONALD H. (See ORLIN BIDDULPH, 182)
- Bud, growth, inhibitory effect of auxin, in guayule, 270
- BURKETT, ALBERT L. (See WILLIAM C. COOPER, 655)
- BURKHOLDER, PAUL R., and EDMUND W. SINNOTT. Morphogenesis of fungus colonies in submerged shaken cultures, 424
- BURR, G. O. (See G. S. RABIDEAU, 349)
- Cabbage, clubroot, plant nutrition in relation to development of, 487; yellows, plant nutrition in relation to disease development, 314
- California, annuals, response to photoperiod and temperature, 1; new marine algae from, 447
- CALYPTRIDIDUM **depressum** sp. nov., 285
- Carbon isotope (C¹³) as a tracer for transport studies in plants, 349
- CASTLE, EDWARD S. The structure of the cell walls of *Aspergillus* and the theory of cellulose particles, 148
- Catenomyces persicinus* Hanson. A morphological, developmental, and cytological study of four saprophytic chytrids, 431
- Cell, elongation, and the development of root hairs in tomato roots, 490; number, in successive segments of *Avena* coleoptiles of different ages, 575; walls, in *Aspergillus*, structure of, in relation to theory of cellulose particles, 148
- Cellulose particles, in *Aspergillus*, theory of, 148
- Chasmogamy, in *Bromus carinatus* Hook. & Arn., 66
- CHEN, SHAO-LIN and P. S. TANG. Studies on colchicine-induced autotetraploid barley. III. Physiological studies, 177; IV. Enzyme activities, 180; SHU-MIN SHEN, and P. S. TANG. Studies on colchicine-induced autotetraploid barley. I. and II. Cytological and morphological observations, 103
- Chiasmata, of the *Allium cepa* x *A. fistulosum* hybrid and its derivatives, 370
- CHILTON, S. J. P., G. B. LUCAS and C. W. EDGERTON. Genetics of *Glomerella*. Crosses with a conidial strain, 549; (See C. W. EDGERTON, 115)

- Chimeras, periclinal, in *Datura* in relation to the development and structure of the ovule, 72
- Chlorella pyrenoidosa*, effect of indole-3-acetic acid on dry weight of, 257; *C. vulgaris*, influence of the age of the culture on the accumulation of chlorellin, 405
- Chlorellin, influence of the age of the culture on the accumulation of, 405
- Chloroplasts, the evolution of oxygen from suspensions of, 291
- CHONDRIA *arcuata* sp. nov., 447
- Chromosome, numbers in higher plants, Acanthaceae to Myrtaceae, 81; Menispermaceae to Verbenaceae, 191
- Chromosomes, of *Neurospora crassa*, 671; production of variable aneuploid numbers within the root tips of *Parphipedium Wardii*, 506
- Chytrid, saprophytic, a morphological, developmental, and cytological study of *Rhizophyidium coronum* Hanson, 479
- Chytrids, Brazilian, observations relative to sexuality in two new species of *Siphonaria*, 580; polycentric species of, 29; *Rhopalophysetis* and *Chytrimyces*, two new chitinophilic operculate genera, 362; saprophytic, study of *Catenomyces*, 431
- Chytrimyces** n. gen. 363; *C. aureus* n. sp. 363; *C. hyalinus* n. sp., 363
- Cleistogamy, in *Bromus carinatus* Hook. & Arn., 66
- Clubroot, of cabbage, plant nutrition in relation to development of, 487
- COGHILL, ROBERT D. (See ALEXANDER HOLLAENDER, 160; LEWIS B. LOCKWOOD, 214; KENNETH B. RAPER, 165)
- Colchicine, in seed germination and early growth, growth stimulation by, 106
- Colchicine-induced, autotetraploid barley, studies on, 103, 177, 180; cytohistological changes in cranberry, 387
- Coleoptiles, *Avena*, of different ages, cell number in successive segments of, 575
- Conidia, of *Glomerella cingulata*, nutrient requirements in germination of, 296
- COOPER, WILLIAM C., ALBERT L. BURKETT and ALEJANDRO HERR. Flowering of Peruvian cube, *Lonchocarpus utilis* A. C. Smith, induced by girdling, 655
- CORMACK, R. G. H. Cell elongation and the development of root hairs in tomato roots, 490
- COSPER, LLOYD. (See F. W. WENT, 643)
- Cotton flowers, growth and phosphorus accumulation in, 182
- COX, L. G. (See ARTHUR J. EAMES, 331)
- Cranberry, mechanism of colchicine-induced cytohistological changes in, 387
- Cryptostegia*, anatomy of, with special reference to latex system, 135; leaf chlorenchyma, rubber in, 619
- Cube, Peruvian, *Lonchocarpus utilis* A. C. Smith, flowering of, induced by girdling, 655
- Cucurbit fruits, relation of size to growth in, 439
- Cucurbita pepo*, calculation of tensions in, 126
- Cultural, characteristics of ultraviolet-induced mutations in *Aspergillus terreus*, 165
- Culture, of tomatoes, in field and air-conditioned greenhouse, 643
- Cuttings, a physiological separation of two factors necessary for the formation of roots on, 336
- Cytogenetic, study of polyembryony, in *Asparagus officinalis* L., 560
- Cytogenetics, of certain *Triticum-Agropyron* hybrids, and their fertile derivatives, 451
- Cytohistological, changes in cranberry, colchicine-induced, 387
- Cytological, study of *Rhizophyidium coronum* Hanson, 479; observations on colchicine-induced autotetraploid barley, 103
- Cytoplasmic, basis of intercellular patterns in vascular differentiation, 151
- Datura*, periclinal chimeras in, in relation to the development and structure of the ovule, 72
- DAWSON, RAY F. An experimental analysis of alkaloid production in *Nicotiana*: The origin of nornicotine, 416
- Deficiencies, of certain mineral elements, effects of, on the development of *Taraxacum kok-saghyz*, 523
- Defoliation, in guayule, and anatomy of leaf abscission, 250
- DELPHINIUM *alatum* sp. nov., 286; *hookeri* nom. nov., 286; *nutans* sp. nov., 286; *ruthae* sp. nov., 287
- DEMEREK, M. (See EVA R. SANSOME, 218; ALEXANDER HOLLAENDER, 226)
- DERMEN, HAIG. The mechanism of colchicine-induced cytohistological changes in cranberry, 387
- Deschampsia caespitosa*, some ecotypic relations of, 298; *D. caespitosa* subsp. *genuina* comb. nov., 302; subsp. *beringensis* comb. nov., 302; subsp. *holciformis* comb. nov., 302
- Desynopsis, in the common wheat, 92
- Development, of sclereids, in the foliage leaf of *Trochodendron aralioides*, 456; of *Taraxacum kok-saghyz*, effects of deficiencies of certain mineral elements on, 523
- Developmental, anatomy, of *Phlox Drummondii* Hook. The embryo, 588; the seedling, 628; study of *Rhizophyidium coronum* Hanson, 479
- Differentiation, in root tip of *Phleum pratense*, 36
- DIMOND, ALBERT E., and GEORGE L. PELTIER. Controlling the pH of cultures of *Penicillium notatum* through its carbon and nitrogen nutrition, 46
- Disease, a tumor-forming virus in plants, 408; plant nutrition in relation to development of cabbage yellows, 314; of cabbage clubroot, 487
- Distribution, geographic, of Verbenaceae and Avicenniaceae, 609
- Dodonaea* L. (fam. Sapindaceae, additions to, 202; *D. ERIOCARPA* β **confertior** var. nov., 206; *D. ERIOCARPA* ι **Degeneri** var. nov. et **Degeneri** f. nov., 208; *D. ERIOCARPA* γ **Forbesii** var. nov., 206; *D. ERIOCARPA* π **minor** var. nov., 212; *D. ERIOCARPA* κ **glabrescens** var. nov., 209; *D. ERIOCARPA* ξ **Hildebrandii** var. nov., 211; *D. ERIOCARPA* η **Hoskana** var. nov., 207; *D. ERIOCARPA* ζ **lanaiensis** var. nov., 207; *D. ERIOCARPA* ν **molokaiensis** Degener & Sherff var. nov., 211; *D. ERIOCARPA* β **oblonga** var. nov., 208; *D. ERIOCARPA* ϵ **obtusior** var. nov. et **obtusior** f. nov., 206; *D. ERIOCARPA* var. ϵ **obtusior galapagensis** f. nov., 207; *D. ERIOCARPA* λ **pallida** Degener & Sherff, var. nov. et f. **pallida** Deg. & Sherff f. nov., 210; *D. ERIOCARPA* var. λ **pallida** f. **acuminatula** Deg. & Sherff, f. nov., 210; *D. ERIOCARPA* var. **typica**, var. nov., 204; *D. ERIOCARPA* μ **Vaccinoides** var. nov., 210; *D. ERIOCARPA* ς **waimeana** var. nov., 206; *D. eriocarpa* σ **Waitziana** (Blume) comb. nov., 212; *D. sandwicensis* sp. nov., 202; *D. SANDWICENSIS* β **simulans** var. nov., 204; *D. stenoptera* var. **Fauriei** (Lévl.) comb. nov. 213; *D. STENOPTERA* **typica** var. nov. 213; *D. viscosa* var. **arborescens** (Cunn.) comb. nov. et f. **arborescens** f. nov., 214; *D. viscosa* var. **arborescens** f. **Ehrenbergii** (Schlecht.) comb. nov., 214; *D. viscosa* var. **arborescens** f. **spatulata** (Sm.) comb. nov., 214; *D. viscosa* var. **linearis** f. **angustifolia** (Benth.) comb. nov., 214; *D. viscosa* var. **linearis** f. **arizonica** (A. Nels.) comb. nov., 214; *D. viscosa* var. **linearis** (Harv. & Sond.) comb. nov. et f. **linearis** f. nov., 214; *D. viscosa minor* var. nov., 212
- DUGGAR, B. M. (See ALBERT C. HILDEBRANDT, 357)
- DUNCAN, ROBERT E. Production of variable aneuploid numbers of

- chromosomes within the root tips of *Paphiopedilum Wardii*, 506
- EAMES, ARTHUR J., and L. G. COX. A remarkable tree-fall and an unusual type of graft-union failure, 331
- Ecotypic relations, of *Deschampsia caespitosa*, 298
- EDGERTON, C. W., S. J. P. CHILTON, and G. B. LUCAS. Genetics of *Glomerella*. II. Fertilization between strains, 115; (See S. J. P. CHILTON, 549)
- Embryo, developmental anatomy of, in *Phlox Drummondii* Hook., 588
- EMSWELLER, S. L., and H. A. JONES. Further studies on the chiasmata of the *Allium cepa* × *A. fistulosum* hybrid and its derivatives, 370
- Endomitotic, tapetal cell divisions in *Spinacia*, 326
- ENGARD, CHARLES J. Habit of growth of *Rubus rosaefolius* Smith in Hawaii, 536
- Enzyme, activities in colchicine-induced autotetraploid barley, 180
- ERICKSON, LOUIS C. The water factor in translating guayule, 634
- ERIGERON *denalii* sp. nov., 289
- ESAU, KATHERINE. Vascularization of the vegetative shoots of *Helianthus* and *Sambucus*, 18
- Excised, stem tips of asparagus, cultivation in vitro, 13; tobacco and sunflower tissue, in vitro growth of, with different temperatures, hydrogen-ion concentrations and amounts of sugar, 357
- Extraction of auxin from green plant tissue, 188
- Exudation, rate of, effect of moisture content of the soil upon, 570
- Fertilization, affect on growth and phosphorus accumulation in cotton flowers, 182; between strains of *Glomerella*, genetics of, 115
- FOSTER, ADRIANCE S. Origin and development of sclereids in the foliage leaf of *Trochodendron aralioides*, 456
- FRENCH, C. S. (See JOHANNA KUMM, 291)
- Fungus, colonies in submerged shaken cultures, morphogenesis of, 424
- Fusarium*, species concept in, with reference to Discolor and other sections, 657; wilt resistance in the Pan American tomato, apparent localization of, 62
- Gametic, reproduction by *Phycomyces*, factor Z_2 and, 320
- Genetic, variation, among apomictically produced plants of several F_1 progenies of guayule (*Parthenium argentatum*) and mariola (*P. incanum*), 554
- Genetics, of *Glomerella*. Crosses with a conidial strain, 549; of *Glomerella*, fertilization between strains, 115
- Geographic, distribution, of Verbenaceae and Avicenniaceae, 609
- Germination, of conidia of *Glomerella cingulata*, nutrient requirements, 296; of seed, stimulation by manganese sulphate, indole-3-acetic acid, and colchicine, 106
- GIGARTINA *tepida* sp. nov., 449
- Girdling, resulting in flowering of Peruvian cube, *Lonchocarpus utilis* A. C. Smith, 655
- Glomerella*, genetics of. Crosses with a conidial strain, 549; genetics of, fertilization between strains, 115; *G. cingulata*, nutrient requirements in germination of conidia, 296
- GOODWIN, RICHARD H., and WILLIAM STEPKA. Growth and differentiation in the root tip of *Phleum pratense*, 36
- GORHAM, PAUL R. Growth factor studies with *Spirodela polyrrhiza* (L.) Schleid., 496
- Graft tumors, metastatic, of bacteria-free crown-galls on *Vinca rosea*, 257
- Graft-union, failure, resulting in a remarkable tree-fall, 331
- GRAY, WILLIAM D. The existence of physiological strains in *Physarum polycephalum*, 157
- Greenhouse, air-conditioned, and field culture of tomatoes, comparison between 643
- GREGORY, LUIS E. (See J. VAN OVERBEEK, 336)
- GRIFFITHSIA MULTIRAMOSA TAYLOR, var. *Balboensis*, var. nov., 447
- Growth, and phosphorus accumulation in cotton flowers as affected by meiosis and fertilization, 182; and vascular development in the shoot apex of *Sequoia sempervirens* (Lamb.) Endl. II. Vascular development in relation to phyllotaxis, 380; factors, for *Trichophyton mentagrophytes*, 509; factors, in *Spirodela polyrrhiza* (L.) Schleid., 496; habit, of *Rubus rosaefolius* Smith in Hawaii, 536; in root tip of *Phleum pratense*, 36; in shoot apex of *Sequoia sempervirens* (Lamb.) Endl., 118; in vitro of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar, 357; of California annuals, under controlled conditions, response to photoperiod and temperature, 1; of plant, under controlled conditions. The relation between age, light, variety and thermoperiodicity of tomatoes, 469; of shoot apex of *Sequoia sempervirens* (Lamb.) Endl., 118; of tomatoes in field and air-conditioned greenhouse 643; relation of, to size in cucurbit fruits, 439; stimulation by manganese sulphate, indole-3-acetic acid, and colchicine, in seed germination and early growth, 106
- Guayule, the water factor in transplanting, 634; and mariola, crosses between, 395; genetic variation among apomictically produced plants in F_1 progenies of, 554
- Hairs, root, development of, in tomato, 490
- HANSON, ANNE M. A morphological, developmental, and cytological study of four saprophytic chytrids. I. *Catenomyces persicinus* Hanson, 431; II. *Rhizophyidium coronum* Hanson, 479
- HANSEN, H. N. (See WILLIAM C. SNYDER, 657)
- HARLAN, JACK R. Cleistogamy and chasmogamy in *Bromus carinatus* Hook. & Arn., 66; Natural breeding structure in the *Bromus carinatus* complex as determined by population analysis, 142
- HEINZE, P. H. and C. F. ANDRUS. Apparent localization of *Fusarium* wilt resistance in the Pan American tomato, 62
- Helianthus*, vascularization of vegetative shoots in, 18
- HERR, ALEJANDRO. (See WILLIAM C. COOPER, 655)
- HETEROSIPHONIA *asymmetria* sp. nov., 449
- HILDEBRANDT, ALBERT C., A. J. RIKER, and B. M. DUGGAR. Growth in vitro of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar, 357
- HOLLAENDER, ALEXANDER, KENNETH B. RAPER, and ROBERT D. COGHILL. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. I. Production of the mutations, 160; Eva R. SANSOME, E. ZIMMER, and M. DEMEREC. Quantitative irradiation experiments with *Neurospora crassa*. II. Ultraviolet irradiation, 226; (See KENNETH B. RAPER, 165; Eva R. SANSOME, 218)
- HOLLENBERG, GEORGE J. New marine algae from southern California, 447
- HOOKE, W. J., (See J. C. WALKER, 314, 487)
- Hybridization, interspecific, in *Parthenium* I. Crosses between guayule (*P. argentatum*) and mariola (*P. incanum*), 395
- Hybrids, between *Oryzopsis hymenoides*, and several species of *Stipa*, 599; *Triticum-Agropyron*, cytogenetics of, 451
- Indole-3-acetic acid, effect on dry weight of *Chlorella pyrenoidosa*, 257; in seed germination and early growth, growth stimulation by, 106

- Inheritance, of tree and flower characters of ornamental edible peaches, 53
- Inhibitory, effect of auxin on bud growth in guayule, 270
- Intercellular, patterns in vascular differentiation, cytoplasmic basis of, 151
- Interspecific, hybridization in *Parthenium* I. Crosses between guayule (*P. argentatum*) and mariola (*P. incanum*), 395
- In vitro growth, of excised tobacco and sunflower tissue with different temperatures, hydrogen-ion concentrations and amounts of sugar, 357
- Irradiation, quantitative, experiments with *Neurospora crassa*, 218, 226; ultraviolet, of *Neurospora crassa*, 226
- JONES, H. A. (See S. L. EMSWELLER, 370)
- JOHNSON, B. LENNART. Natural hybrids between *Oryzopsis hymenoides* and several species of *Stipa*, 599
- JUDKINS, WESLEY P. The extraction of auxin from tomato fruit, 242
- KARLING, JOHN S. Brazilian chytrids. V. *Nowakowskiella macrospora* n. sp., and other polycentric species, 29; Brazilian chytrids. VI. *Rhopalophlyctis* and *Chytriumyces*, two new chitinophyllic operculate genera, 362; Brazilian chytrids. VII. Observations relative to sexuality in two new species of *Siphonaria*, 580
- KELNER, ALBERT. (See ROBERT T. WHITTENBERGER, 619)
- KUMM, JOHANNA and C. S. FRENCH. The evolution of oxygen from suspensions of chloroplasts; the activity of various species and the effects of previous illumination of the leaves, 291
- LAMMERTS, WALTER E. The breeding of ornamental edible peaches for mild climates. I. Inheritance of tree and flower characters, 53
- Latex system, in *Cryptostegia grandiflora*, 135
- LAWRENCE, WILLIAM E. Some ecotypic relations of *Deschampsia caespitosa*, 298
- Leaf, abscission, anatomy of, and experimental defoliation in guayule, 250; of *Trochodendron aralioides*, origin and development of schlereids in, 456
- Leaves, auxin in, inhibitory effect on bud growth in guayule, 270
- LEWIS, HARLAN, and F. W. WENT. Plant growth under controlled conditions. IV. Response of California annuals to photoperiod and temperature, 1
- LI, C. H. (See H. W. LI, 92)
- LI, H. W., W. K. PAO, and C. H. LI. Desynopsis in the common wheat, 92
- Light, relation to age, variety and thermo-periodicity in tomatoes, 469
- LIN, CH'WAN-KWANG. Nutrient requirements in the germination of the conidia of *Glomerella cingulata*, 296
- Local-lesion method, accuracy of, for measuring activity of southern bean mosaic virus, 613
- LOCKWOOD, LEWIS B., KENNETH B. RAPER, ANDREW J. MOYER and ROBERT D. COGHILL. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. III. Biochemical characteristics of the mutations, 214
- Lonchocarpus utilis* A. C. Smith, flowering of, induced by girdling, 655
- LOO, SHIH-WEI. Cultivation of excised stem tips of asparagus in vitro, 13
- LOO, TSUNG-LÉ, and YÜ-WEI TANG. Growth stimulation by manganese sulphate, indole-3-acetic acid, and colchicine in the seed germination and early growth, 106
- LOVE, R. MERTON, and C. A. SUNESON. Cytogenetics of certain *Triticum-Agropyron* hybrids and their fertile derivatives, 451
- LUCAS, G. B. (See S. J. P. CHILTON, 549; C. W. EDGERTON, 115)
- LUPINUS *toklatensis* sp. nov., 288
- McCLINTOCK, BARBARA. Preliminary observations of the chromosomes of *Neurospora crassa*, 671
- McDERMOTT, J. JOSEPH. The effect of the moisture content of the soil upon the rate of exudation, 570
- MA, ROBERTA. (See WILLIAM J. ROBBINS, 509)
- Manganese sulphate, in seed germination and early growth, growth stimulation by, 106
- MANTON, IRENE. New evidence on the telophase split in *Todea barbara*, 342
- Marine, algae, from southern California, 447
- Mariola (*Parthenium incanum*) and guayule (*P. argentatum*), crosses between, 395; genetic variation among apomictically produced plants in F₁ progenies of, 554
- Meiosis, affect on growth and phosphorus accumulation in cotton flowers, 182
- Menispermaceae, chromosome numbers in, 191
- Metastatic, graft tumors of bacteria-free crown-galls on *Vinca rosea*, 237
- MEYER, BERNARD S. Effects of deficiencies of certain mineral elements on the development of *Taraxacum kok-saghyz*, 523
- MILLER, HELENA A. and RALPH H. WETMORE. Studies in the developmental anatomy of *Phlox Drummondii* Hook. I. The embryo, 588; II. The seedling, 628
- Mineral, elements, effects of deficiencies of, on the development of *Taraxacum kok-saghyz*, 523
- Mineral salts, KH₂PO₄, M₂SO₄, and NaNO₃, influence of the proportions of, on the production of penicillin in surface cultures, 528
- Moisture, content, of the soil, effect of, upon the rate of exudation, 570
- MOLDENKE, HAROLD N. The known geographic distribution of the members of the Verbenaceae and Avicenniaceae, 609
- Monoblepharella*, morphologic study of, 259
- Morphogenesis, of fungus colonies in submerged shaken cultures, 424
- Morphologic, study of the genus *Monoblepharella*, 259
- Morphological, characteristics of ultraviolet-induced mutations of *Aspergillus terreus*, 165; developmental, and cytological study of four saprophytic chytrids. I. *Catenomyces persicinus* Hanson, 431; II. *Rhizophyidium coronum* Hanson, 479; observations on colchicine-induced autotetraploid barley, 103
- Mosaic, of bean, accuracy of the local-lesion method for measuring virus activity of, 613
- MOYER, ANDREW J. (See LEWIS B. LOCKWOOD, 214)
- Mutations, concerned with nutritional requirements, methods of producing and detecting in *Neurospora*, 678; in *Aspergillus terreus*, induced by ultraviolet, 160, 214
- MYRIOGRAMME *repens* sp. nov., 449
- NELSON, AVEN. Rocky Mountain herbarium studies. VI. 284
- Neurospora*, methods of producing and detecting mutations concerned with nutritional requirements in, 678; *N. crassa*, chromosomes of, 671; quantitative irradiation experiments with, 218, 226
- Nicotiana*, analysis of alkaloid production in: The origin of nornicotine, 416
- Nitrogen, and auxin, relationships in green plants, 666; polyploidy and auxin in green plant tissue, 669
- Nornicotine, origin of, an experimental analysis of alkaloid production in *Nicotiana*, 416
- NOWAKOWSKIELLA *macrospora* n. sp., 29
- Nutrient, requirements in the germination of conidia of *Glomerella cingulata*, 296

- Nutrition, carbon and nitrogen, control of pH of cultures of *Penicillium notatum* through, 46; in relation to disease development. I. Cabbage yellows, 314; II. Cabbage clubroot, 487
- Nutritional requirements, methods of producing and detecting mutations concerned with, in *Neurospora*, 678
- ONETO, JOHN F. (See ROBERTSON PRATT, 405)
- Oryzopsis hymenoides*, natural hybrids with several species of *Stipa*, 599
- Oryzopsis hymenoides* × *Stipa Elmeri***, *hyb. nov.*, 605; × ***Stipa californica***, *hyb. nov.*, 607; × ***Stipa Scribneri***, *hyb. nov.*, 607; × ***Stipa robusta*** *hyb. nov.*, 607; × ***Stipa columbiana***, *hyb. nov.*, 608
- OVERBEEK, J. VAN, and LUIS E. GREGORY. A physiological separation of two factors necessary for the formation of roots on cuttings, 336
- Ovule, periclinal chimeras in *Datura* in relation to the development and structure of, 72
- Oxygen, evolution of, from suspensions of chloroplasts; activity of various species and effects of previous illumination of the leaves, 291
- PAO, W. K. (See H. W. LI, 92)
- Paphiopedilum Wardii*, production of variable aneuploid chromosome numbers in root tips of, 506
- Parthenium argentatum* (guayule) and *P. incanum* (mariola), crosses between, 395; genetic variation among apomictically produced plants in F_1 progenies of, 554
- Peaches, ornamental edible, inheritance of tree and flower characters in, 53
- PELTIER, GEORGE L. (See ALBERT E. DIMOND, 46)
- Penicillin, in surface cultures, influence of the proportions of KH_2PO_4 , $MgSO_4$ and $NaNO_3$ on the production of, 528
- Pencillium notatum*, control of pH of cultures of, through carbon and nitrogen nutrition, 46
- Peruvian, cube, *Lonchocarpus utilis* A. C. Smith, flowering of, induced by girdling, 655
- PETROGLOSSUM ***parvum*** *sp. nov.*, 450
- pH, of cultures of *Pencillium notatum*, control of, through carbon and nitrogen nutrition, 46
- Phleum pratense*, growth and differentiation in root tip of, 36
- Phlox Drummondii* Hook., developmental anatomy of. The embryo, 588; the seedling, 628
- Phosphorus accumulation, and growth in cotton flowers as affected by meiosis and fertilization, 182
- Photoperiod, response of California annuals to, 1
- Phycomyces*, factor Z_2 and gametic reproduction by, 320
- Phyllotaxis, vascular development in relation to, 380
- Physarum polycephalum*, physiological strains in, 157
- Physiological, separation of two factors necessary for the formation of roots on cuttings, 336; strains in *Physarum polycephalum*, 157; studies on colchicine-induced autotetraploid barley, 177
- PIPER, MARGARET. (See G. S. AVERY, JR., 575)
- Polyembryony, in *Asparagus officinalis* L., cytogenetics of, 560
- Polyploidy, auxin and nitrogen in green plant tissue, 669
- Population analysis, in *Bromus carinatus*, 142
- PORPHYRELLA ***californica*** *sp. nov.*, 450
- POTTORF, LOUISE. (See GEORGE S. AVERY, JR., 666, 669)
- PRATT, JANE. (See ROBERTSON PRATT, 405)
- PRATT, ROBERTSON. Influence of the proportions of KH_2PO_4 , $MgSO_4$ and $NaNO_3$ in the nutrient solution on the production of penicillin in surface cultures, 528; JOHN F. ONETO, and JANE PRATT. Studies on *Chlorella vulgaris*. Influence of the age of the culture on the accumulation of chlorellin, 405
- PRICE, W. C. Accuracy of the local lesion method for measuring virus activity. IV. Southern bean mosaic virus, 613
- PRIESTLEY, J. H. Observations on spiral grain in timber, 277
- Quantitative irradiation experiments with *Neurospora crassa*, 218, 226
- RABIDEAU, G. S., and G. O. BURR. The use of the C^{13} isotope as a tracer for transport studies in plants, 349
- RANDALL, THOMAS E., and CHARLES M. RICK. A cytogenetic study of polyembryony in *Asparagus officinalis* L., 560
- RAPER, KENNETH B., ROBERT D. COGHILL, and ALEXANDER HOLLAENDER. The production and characterization of ultraviolet-induced mutations in *Aspergillus terreus*. II. Cultural and morphological characteristics of the mutations, 165; (See ALEXANDER HOLLAENDER, 160; LEWIS B. LOCKWOOD, 214)
- Resistance, to *Fusarium* wilt, in the Pan American tomato, 62
- Rhizophydium coronum* Hanson, a morphological, developmental, and cytological study of, 479
- Rhopalophlyctis*** *n. gen.*, 363; *R. sarcoptoides* *n. sp.* 363
- RICK, CHARLES M. (See THOMAS E. RANDALL, 560)
- RIKER, A. J. (See ALBERT C. HILDEBRANDT, 357)
- ROBBINS, WILLIAM J., and ROBERTA M. A. Growth factors for *Trichophyton mentagrophytes*, 509; and MARY BARTLEY SCHMITT. Factor Z_2 and gametic reproduction by *Phycomyces*, 320
- Rocky Mountain, herbarium studies, 284
- ROLLINS, REED C. Evidence for genetic variation among apomictically produced plants of several F_1 progenies of guayule (*Parthenium argentatum*) and mariola (*P. incanum*), 554; Interspecific hybridization in *Parthenium* I. Crosses between guayule (*P. argentatum*) and mariola (*P. incanum*), 395
- Root, hairs, development of, in tomato, 490; tip, of *Phleum pratense*, growth and differentiation in, 36; tips, of *Paphiopedilum Wardii*, production of variable aneuploid chromosome numbers, 506
- Roots, a physiological separation of two factors necessary for the formation of, on cuttings, 336
- Rubber, in *Cryptostegia* leaf chlorenchyma, 619
- Rubus rosaefolius* Smith, habit of growth of, 536
- Rye, and sunflower, silicon absorption by, 539
- Sambucus*, vascularization of vegetative shoots in, 18
- SANSOME, EVA R., M. DEMEREC and ALEXANDER HOLLAENDER. Quantitative irradiation experiments with *Neurospora crassa*, 218; (See ALEXANDER HOLLAENDER, 226)
- Sapindaceae, some additions to the genus *Dodonaea* L., 202
- Saprophytic chytrids, morphological, developmental, and cytological study of *Catenomyces*, 431
- SATINA, SOPHIE. Periclinal chimeras in *Datura* in relation to the development and structure of the ovule, 72
- SCHMITT, MARY BARTLEY. (See WILLIAM J. ROBBINS, 320)
- Sclereids, development of, in the foliage leaf of *Trochodendron aralioides*, 456
- Seed, germination and early growth, stimulation by manganese sulphate, indole-3-acetic acid, and colchicine, 106
- Seedling, of *Phlox Drummondii*, developmental anatomy of, 628
- SELL, HAROLD MELVIN (See M. A. BRANNON, 257)
- SENECIO ***denalii*** *sp. nov.*, 289

- Sequoia sempervirens* (Lamb.) Endl. growth and vascular development in shoot apex of, 118, 380
- Sexuality, observations relative to, in two new species of *Siphonaria*, 580
- SHEN, SHU-MIN (See SHAO-LIN CHEN, 103)
- SHERFF, EARL EDWARD. Some additions to the genus *Dodonaea* L. (fam. Sapindaceae), 202
- Shoot apex, *Sequoia sempervirens* (Lamb.) Endl., growth and vascular development in, 118, 380
- Shoots, vegetative, of *Helianthus* and *Sambucus*, vascularization in, 18
- Silicon, absorption, by rye and sunflower, 539
- SINNOTT, EDMUND W. The relation of growth to size in cucurbit fruits, 439; and ROBERT BLOCH. The cytoplasmic basis of intercellular patterns in vascular differentiation, 151; (See PAUL R. BURKHOLDER, 424)
- Siphonaria*, two new species of, observations relative to sexuality, 580; *S. petersonii* sp. nov., 580; *S. sparrowii* sp. nov., 581
- SMITH, PATRICIA. (See G. S. AVERY, JR., 575)
- SMITH, PAUL F. Auxin in leaves and its inhibitory effect on bud growth in guayule, 270
- SNYDER, WILLIAM C. and H. N. HANSEN. The species concept in *Fusarium* with reference to Discolor and other sections, 657
- Soil, effect of the moisture content of, upon the rate of exudation, 570
- Species, concept, in *Fusarium* with reference to Discolor and other sections, 657
- Spinacia*, endomitotic tapetal cell divisions in, 326
- Spiral grain, in timber, 277
- Spirodela polyrrhiza*, growth factors in, 496
- SPRINGER, MARTHA E. A morphologic study of the genus *Monoblepharella*, 259
- Stem, excised tips of asparagus, cultivation in vitro, 13
- STEPKA, WILLIAM (See RICHARD H. GOODWIN, 36)
- STERLING, CLARENCE. Growth and vascular development in the shoot apex of *Sequoia sempervirens* (Lamb.) Endl. I. Structure and growth of shoot apex, 118; II. Vascular development in relation to phyllotaxis, 380
- Stipa*, natural hybrids with *Oryzopsis hymenoides*, 599
- X *Stiporyzopsis Bloomeri* comb. nov., 602
- STOCKING, C. RALPH. The calculation of tensions in *Cucurbita pepo*, 126
- Structure, cell walls of *Aspergillus*, and theory of cellulose particles, 148; of shoot apex of *Sequoia sempervirens* (Lamb.) Endl., 118
- SUNESON, C. A. (See R. MERTON LOVE, 451)
- Sunflower, and rye, silicon absorption by, 539; excised tissue of, growth in vitro, with different temperatures, hydrogen-ion concentrations and amounts of sugar, 357
- TANG, P. S. (See SHAO-LIN CHEN, 103, 177, 180)
- TANG, YÜ-WEI (See TSUNG-LÉ Loo, 106)
- Tapetum, endomitotic cell divisions in, of *Spinacia*, 326
- TARAXACUM *carneocoloratum* sp. nov., 290
- TATUM, E. L. (See G. W. BEADLE, 678)
- Telophase, split in *Todea barbara*, 342
- Temperature, response of California annuals to, 1
- Tensions, in *Cucurbita pepo*, calculation of, 126
- Thermo-periodicity, relation to age, light and variety in tomatoes, 469
- THLASPI *australe* sp. nov., 287; *prolixum* sp. nov., 287; *stipitatum* sp. nov., 288
- Timber, spiral grain in, 277
- Tobacco, excised tissue, growth in vitro of, with different temperatures, hydrogen-ion concentrations and amounts of sugar, 357
- Todea barbara*, telophase split in, 342
- Tomato; fruit, extraction of auxin from, 242; roots, cell elongation and development of root hairs in, 490
- Tomatoes, comparison between field and air-conditioned greenhouse culture of, 643; relation between age, light, variety and thermo-periodicity in, 469
- Tracer, C¹³ isotope as, for transport studies in plants, 349
- Transplanting, of guayule, water as a factor in, 634
- Transport, studies in plants, use of C¹³ isotope as tracer for, 349
- Tree-fall, and an unusual type of graft-union failure, 331
- Trichophyton mentagrophytes*, growth factors for, 509
- Triticum-Agropyron*, hybrids, cytogenetics of, and their fertile derivatives, 451
- Trochodendron aralioides*, origin and development of sclereids in the foliage leaf, 456
- Tumor, disease of plants, due to virus, 408
- Tumors, metastatic graft, of bacteria-free crown-galls on *Vinca rosea*, 237
- Ultraviolet, irradiation of *Neurospora crassa*, 226
- Ultraviolet-induced, mutations in *Aspergillus terreus*, 160, 165, 214
- Vascular development, and growth, in the shoot apex of *Sequoia sempervirens* (Lamb.) Endl., 118, 380; differentiation, cytoplasmic basis of intercellular patterns in, 151
- Vascularization, of vegetative shoots of *Helianthus* and *Sambucus*, 18
- Verbenaceae, and Avicenniaceae, geographic distribution of, 609
- Vinca rosea*, metastatic (graft) tumors of bacteria-free crown-galls on, 237
- Virus, activity, accuracy of the local-lesion method for measuring, 613; tumor disease of plants, 408
- WALKER, J. C., and W. J. HOOKER. Plant nutrition in relation to disease development. I. Cabbage yellows, 314; II. Cabbage clubroot, 487
- Water, a factor in transplanting guayule, 634
- WENT, F. W. Plant growth under controlled conditions V. The relation between age, light, variety and thermo-periodicity of tomatoes, 469; and LLOYD COSPER. Plant growth under controlled conditions VI. Comparison between field and air-conditioned greenhouse culture of tomatoes, 643; (See HARLAN LEWIS, 1)
- WETMORE, RALPH H. (See HELENA A. MILLER, 588, 628)
- Wheat, desynapsis in, 92
- WHITE, PHILIP R. Metastatic (graft) tumors of bacteria-free crown-galls on *Vinca rosea*, 237
- WHITE, R. O. (See G. S. AVERY, JR., 188)
- WHITTENBERGER, ROBERT T. Silicon absorption by rye and sunflower, 539; and ALBERT KELNER. Rubber in *Cryptostegia* leaf chlorenchyma, 619
- Wilt, *Fusarium*, localization of resistance to, in the Pan American tomato, 62
- WIRKUS, E. R. Endomitotic tapetal cell divisions in *Spinacia*, 326
- X-rays, quantitative irradiation experiments with, using *Neurospora crassa*, 226
- Z₂, and gametic reproduction by *Phycomyces*, 320
- ZIMMER, E. (See ALAXANDER HOLLAENDER, 226)